PARASITOLOGY

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PARASITOLOGY

EDITED BY

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EDWARD HINDLE, PH.D.

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SPIROCHAETA ICTEROHAEMORRHAGIAE IN THE COMMON RAT IN ENGLAND.

WITH REMARKS ON THE MINUTE STRUCTURE OF THESE LEPTOSPIRA (NOGUCHI).

BY ALFRED C. COLES, M.D., D.Sc., F.R.S.E., M.R.C.P. (LOND.).

Physician to the Royal National Sanatorium, Bournemouth.

(With Plate I.)

THE Japanese observers Ido, Hoki, Ito and Wani (1916) state that in Infectious Jaundice the infective organism—the *Spirochaeta icterohaemorrhagiae*—is transmitted probably from rats to man by means of the urine of rats, directly or indirectly. They say:

"We have carefully examined house and ditch rats in the city and rats in the coal mines where Weil's Disease prevails, and found 39·5 % contain highly virulent pathogenic spirochaetes in their kidneys.

"The kidneys were examined microscopically under dark ground illumination, and in cases in which we did not find the pathogenic spirochaetes we made inoculations into guinea-pigs.

"Thus we found *S. icterohaemorrhagiae* microscopically in kidney or urine in 32·4 %, by inoculation in 7 %, making a total of 39·5 % out of a total of 86 rats."

This autumn the same workers (1917) state: "In Japan the rat is undoubtedly a carrier of the causal agent of spirochaetosis icterohaemorrhagica. *Mus decumanus* was found to be the carrier in 40·2 % of 149 cases, *Mus alexandrinus* in 0·8 % of 24 cases.

"In 34 out of 92 cases or 37 % spirochaetes were present in the kidney or urine. The organisms cannot be demonstrated in the blood and liver, but in the urine of rats harbouring *S. icterohaemorrhagiae* in the kidney, they were present without exception."

Finally they state: "On the basis of these findings we conclude that 1. The extermination of rats and field mice is a highly important prophylactic measure against Weil's Disease. 2. The chemical composition of the soil and water plays an important part in the development of *S. icterohaemorrhagiae* and consequently in the spread of the disease of which it is the causal agent."

In France, Adrian Stokes found much the same thing. In an after note to his paper (1917) he says: "Since writing the paper we have been able to confirm the statement of the Japanese workers mentioned in the paper with

regard to the finding of the spirochaetae in the kidneys of field rats. Of 9 rats taken from the right part of Segment 1 in the chart in the paper, 5 proved infective to guinea-pigs. Of 6 rats from the left end of Segment 1 one communicated the disease. The kidney of the rat was crushed and emulsified, and injected intra-peritoneally into the guinea-pig. The disease in the infected animal was typical, and we have found the typical *S. icterohaemorrhagiae* in the organs of the guinea-pig. Levaditi preparations of the rat kidney have shown the spirochaetae, and we have also found them in films made from an emulsion of the kidney stained by Fontana's stain."

Martin and Pettit (1917) by inoculating an emulsion of the liver, spleen, and suprarenal body of rats, taken in the war area, into guinea-pigs produced all the symptoms of spirochaetosis icterohaemorrhagica.

In America, Noguchi (1917) had similar results. He says: "We have collected a large number of wild rats in this country (the vicinity of New York City) and removed their kidneys for purpose of ascertaining whether or not the organs contained the spirochaete which causes the typical experimental lesions characteristic of the organism of infective jaundice." "By inoculating the emulsion made of the kidneys of 41 wild rats into 58 guinea-pigs during the last 3 months, we have been able to produce in three groups of guinea-pigs (four in each group) a typical icterohaemorrhagic spirochaetosis altogether identical with the findings in the guinea-pigs which died of the injections of the Japanese and Belgian strains of *S. icterohaemorrhagiae*.

Since it was our practice to make an emulsion from the kidneys (eight) of four wild rats and inject the same into four to six normal guinea-pigs, it is difficult to say whether the icterohaemorrhagic spirochaetosis produced in some of the guinea-pigs in each group was due to one or more rats employed. It would have been better if we had inoculated several guinea-pigs with the emulsion of kidneys from each rat, but this would have involved a large number of guinea-pigs, and hence our method of mixing four in each group. Since the successful inoculation of the organisms from three of the pooled groups, we have resorted to individual tests, the results of which will be reported later."

Jobling and Eggstein, at the 11th Annual Meeting of the Southern Medical Association held in Memphis, Tenn. U.S.A. on Nov. 12th, 1917, stated "that their work had shown that hemorrhagic icterus was present in their community, and that about 10 % of the wild rats harbored the causative organisms." "It was possible that we now saw the evolution of a new epidemic disease."

Nicolle and Blanc (1917), in Tunis, between Feb. 9th and March 6th, examined 91 *Mus decumanus* for *S. icterohaemorrhagiae* and 28 for the organisms of rat-bite fever. Guinea-pigs were inoculated, and ultra microscopic examinations were made in every case, but the results were invariably negative. The writers state that these 110 rats were obtained from the streets of the town and not from the sewers or the vicinity of the port and do not conclude that these infections are absent because of their negative results.

During October 1916, thanks to the kindness of Capt. Adrian Stokes, R.A.M.C., I received many films of blood and liver taken from guinea-pigs inoculated with the blood of cases of Infectious Jaundice occurring in France, and in this way I became familiar with the spirochaete. Later he sent me eight films of blood taken from a soldier on the 4th day of the disease. I spent more than a fortnight examining these but entirely failed to find a single spirochaete.

Out of six films sent subsequently from a case (Corpl. MacG.) on the 2nd day of the disease, after numerous very prolonged examinations I found two definite spirochaetes in one film. These I have included in the photomicrographs which I had the pleasure of taking for his paper. Dr G. T. Western has recently sent me a series of blood and liver films, unfixed and unstained, from guinea-pigs inoculated from cases of Weil's Disease. I take the opportunity of expressing here my thanks to both these workers.

When I learnt that Capt. Stokes, like the Japanese observers, had found pathogenic *S. icterohaemorrhagiae* in the kidneys of rats caught in the trenches, I wondered whether any of the common rats in England would also harbour them, so I examined at different times during the year, the kidneys of 14 rats that had been caught in the neighbourhood, but with no success, although I carefully centrifuged the emulsion before making a microscopic examination. Then Noguchi's paper appeared and I started again and examined another five rats but still without success.

However, on November 1st, a half grown common rat, just killed and very much mauled, was brought to me from the immediate neighbourhood, and in the emulsion made from the kidneys I found numerous typical actively moving spirochaetes.

METHODS.

The kidneys are taken from the body of the rat, and the surrounding fat and capsule carefully removed. They are then placed in a small sterile vessel and a few drops of freshly boiled and cooled distilled water or normal saline added and the kidneys are cut up in very small pieces and a thick emulsion made.

From a drop of this emulsion very thin films are prepared and are allowed to dry in the air. When quite dry a few drops of distilled water, previously made slightly alkaline, are poured over the surface and allowed to act for about 30 seconds. They are then stained in Giemsa, without any fixation, for three to four hours. Two stained films and two fresh coverglass preparations were examined by dark ground illumination in every case.

It is of the greatest importance that both the films and fresh preparations should be as thin as possible, otherwise it is easy to overlook the spirochaetes.

Throughout this investigation I have relied entirely on the method of dark ground illumination which I have been using for the last six or seven years for the detection of *S. pallida* in particular and other spirochaetes in general, and which I (1915) have described in detail elsewhere. Those experts

who have seen my method will vouch for the truth of my statement that "the large field of vision, the ease with which a slide can be thoroughly examined, the absence of any eye strain, and above all the remarkable way in which the spirochaete (*S. pallida*) strikes the eye, have only to be seen to be appreciated."

It is enough to say here that I use for this purpose an ordinary paraffin lamp with an $\frac{1}{2}$ inch wick, a small bulls eye, and an achromatic condenser used dry, with a central stop which can be rotated in or out of position at will, so that one can pass from dark ground to ordinary illumination, without even removing the eye from the microscope. I use an 8 mm. ($\frac{1}{3}$ inch) apo. Leitz and generally search with compensating ocular 6, passing if need be to ocular 8, 12, or 18. In the case of fresh coverglass preparations the tube length of the microscope is about that for which the objective is corrected, viz. 170 mm. In the case of stained films I use no coverglass, and, to compensate for this, increase the tube length to 200 mm.

For the detection of *S. pallida* or other spirochaetes in blood I examine the preparation dry, without a coverglass, but as the kidney emulsion is so granular this is impracticable, so in the latter case I smear over the surface of the stained film with liquid paraffin, and let this drain off before making the examination.

It will be seen that the condenser is not oiled to the slide, and hence one is not troubled by the oil running, or the stage becoming greasy. *S. icterohaemorrhagiae* can be detected, with practice, at a magnification of only about 220 diameters, *i.e.* when the No. 6 eyepiece is used, but it is often necessary to confirm it by placing in either the compensating 12 or 18 eyepiece, in which case one obtains a magnification of about 400 to 600 diameters with the 8 mm. objective.

SPIROCHAETA ICTEROHAEMORRHAGIAE IN KIDNEY OF RATS.

The kidneys of 100 common rats (*Mus decumanus*) killed in Bournemouth or in the surrounding country within an area of about eight miles were examined. The rats were taken indiscriminately and brought to me, generally one or two at a time. Nine of them contained *S. icterohaemorrhagiae* as follows:

Total number of *Mus decumanus* examined 100

			Negative	Positive
Town rats	38		36	2
Country rats	62	A. private estate	11	6
		B. farms (piggeries)	33	1
		C. farms, etc.	6	0
		D. farms, etc.	5	0
	100		91	9

The letters A, B, C, D signify different country areas.

What is most striking is that the rats killed on Area A should show such a high proportion, *i.e.* six out of 17, of positives, whilst from Area B, in

another direction, the proportion was but one out of 34 although the latter district contained many piggeries, etc.

The number of spirochaetes found in the emulsion of the kidneys of the positive nine rats varied very considerably. In the first positive rat from the town they were very numerous, as many as three to eight spirochaetes being found in one field and many in groups, whilst in the next positive rat from the same spot only very few were found in a whole preparation. In four of the positive rats from the country they were moderately numerous, whilst in the two others there were only three or four spirochaetes found in stained or fresh films. In the last rat examined large masses of spirochaetes were found.

The length of each rat examined was noted, and I am of the opinion that spirochaetes are more likely to be found in the half grown, in rats of about seven to eight inches long, than in very large or very small rats. My reasons for forming this opinion are that all the positive rats were about this size, and secondly it is reasonable to suppose that infectious diseases generally begin in rats, as they do for the most part in human beings, in young life, and by the time the rat has developed immune bodies and the spirochaetes are only limited to the kidney, it will not be more than half grown. Anyhow, working on this theory, I was able to find *Trypanosoma lewisi* in all stages of development in six out of 100 young rats which I examined during June and July 1913, whilst in the more mature and fully grown rats only the fully developed stage of trypanosomes were found.

MORPHOLOGY OF *SPIROCHAETA ICTEROHAEMORRHAGIAE* FOUND IN RATS.

The spirochaetes found in the emulsion made from the kidney of rats do not always show movement. In one case, although the organisms were fairly numerous and could be definitely recognised by their characteristic shape, there were no signs of any activity, possibly owing to the time that had elapsed after the death of the rat, some 18 to 20 hours, but more probably owing to changes in the kidney. In most cases the distinctive side-to-side movements, of one or both ends, the central portion remaining rigid, were clearly seen, but in no case did I see much movement from place to place.

In the fresh unstained conditions the majority of the parasites showed little or no internal structure, but in many granules could be seen, whilst in a few the spirochaete looked like a row of beads slowly waving about.

Stokes says: "With dark ground illumination we have been disappointed although the apparatus we use—a Leitz stage and a small arc lamp—shows *S. pallida* very well. With dark ground illumination they are much less refractile than *S. pallida* or *S. refringens*." This has not been my experience, I see these spirochaetes more readily than *S. pallida*.

In the Giemsa stained preparations the rat spirochaetes stain a reddish colour, and in size, shape, and particularly in the recurved fish-hook-like ends, they were morphologically identical with *S. icterohaemorrhagiae* met with in

the blood or liver of guinea-pigs inoculated with blood from cases of Weil's Disease. Unfortunately I am not able to say whether they were pathogenic, but I think there can be little doubt about this.

As to the finer morphological characteristics, Noguchi, describing the spirochaetes found in the rats in New York, says:

"The morphology of the organisms corresponds with that of the Japanese and Belgian strains with which we compared it." The Belgian strain was received from Dr Adrian Stokes.

"Its elementary structure is that of a closely wound slender cylindrical thread with tapering ends, averaging 9μ by $0{\cdot}25\mu$. The number of coils is greater in a given length than that of any spirochaete hitherto known. It is so closely wound that within 5μ there are 10 to 12 coils. Near the extremities the coils become closer. They are never very deep, and in general, the aspect of the whole body is that of a transversely barred chain of streptococci. The winding is rarely seen distinctly, although it can be brought out well by a carefully fixed, stained preparation (osmic acid vapour and Giemsa stain) or under powerful dark field illumination. It should be noted that the description of the organism by most authors leaves this point unclear, and so far no satisfactory microphotograph has been reproduced."

When I read this description in the earlier part of last year I at once re-examined the numerous stained preparations I had of the spirochaetes from the blood and liver of inoculated guinea-pigs, but I could not satisfy myself that these organisms showed the structure Noguchi had described. Some months later when I found these spirochaetes in the kidney of rats, I saw the minute spirals in many without any difficulty and subsequently found them in some guinea-pig preparations which had not been fixed before staining.

The secret, in my opinion, of a successful preparation is not so much in the method of staining as in the absence of fixing.

If for example two blood films from an inoculated guinea-pig be air-dried, and if when thoroughly dry one be fixed in absolute alcohol (10 minutes or more), and the other be moistened for a few minutes with distilled water and then both be stained in Giemsa's solution (preferably made with faintly alkaline distilled water) for the same time say two, three, or four hours, the fixed film will probably not show the minute spirals whilst the unfixed film will exhibit them very distinctly at least in parts of the film.

I have found these spirals in some of the spirochaetes from each of the positive cases, and if in doubt about a spirochaete am not satisfied until this structure is found in some of them.

The spirals vary, some are coarse and easily seen, others are finer and more closely packed together, but in all cases they become closer and less evident towards the extremities of the spirochaete. They are seen with a magnification of 800 to 1000 distinctly.

Noguchi writes: "The part of the body which forms the hook termination

is a fine point, but no minute flagellum-like projection could be demonstrated by staining (Loeffler, Pitfield, Casares-Gil, Fontana, etc.) or by dark ground illumination. It is devoid of a terminal filament such as is characteristic of a *Spironema* or *Treponema* and is resistant to saponin (10 %) unlike all other spirochaetes. It calls for a new genus, and on account of its fine and minute windings the name *Leptospira* is suggested."

I found that the fine spirals could be seen in preparations made by mixing a small drop of the kidney emulsion with congo red and then treating the dry film with 1 % HCl alcohol, a method described by Benian (1916), but I seldom obtain a good preparation of any spirochaete with this procedure.

The photomicrographs (Pl. I) at various magnifications, illustrate the spirochaetes in the blood of the experimentally inoculated guinea-pig and in the kidney of naturally infected common English rats. Here the minute spirals can be clearly seen. That the rat spirochaetes are morphologically identical with the *S. icterohaemorrhagiae* of the guinea-pig, there can be no question, as the photographs were taken at precisely the same magnification and under absolutely the same conditions, and even on the same quarter-plate.

In Japan, in Flanders and in America, both in New York and quite recently in the Southern States, pathogenic spirochaetes have been found in the kidney of the ordinary rat, and in all these countries cases of Infectious Jaundice are known to occur.

Now in England I find identically similar spirochaetes in 9 % of the rats killed in the town and surrounding country. It is highly improbable that these rats have only recently become infected by soldiers who have returned home convalescent from Weil's Disease, as some of the infected rats came from remote country villages. The question naturally suggests itself: does so-called Weil's Disease or Infectious Jaundice occur in England?

The articles dealing with the disease in Allbutt's *System*, the *Encyclopaedia Medica*, Osler and Taylor's *Medicine*, and the more recent communications in the Medical Journals do not definitely state the fact, but all agree in associating the disease with insanitary surroundings and with especially such occupations as slaughtermen, sewermen, butchers, etc., which are just those in which the rat comes in the vicinity of man.

Spirochaeta morsus muris.

During the whole of the investigations on rat spirochaetes I have not only searched for *S. icterohaemorrhagiae*, but other forms have been looked for, especially that which the Japanese workers have now proved conclusively is the cause of rat-bite fever. But it was not till I examined the kidneys of the 84th rat, one sent in from the country district B, a half grown rat measuring 7½ inches, that I met with a short, rigid, and thick spirochaete which I have little doubt may be the spirochaete of rat-bite fever.

I have measured 65 spirochaetes and find the average length to be 5·45μ with 2·2 curves. Two types were seen, one a shorter spirochaete *ca.* 4 to 5μ

long with 2 to $2\frac{1}{2}$ turns, these were by far the more numerous, and the other a longer, looser organism measuring 8 to 10μ with only two curves. The latter only constituted about 8 per cent. of the spirochaetes present. It is possible that some are shorter even than 4μ but I am at present uncertain about some very short organisms.

Futaki, Takaki, Taniguchi and Osumi (1916, 1917), who discovered them first in the excised skin and lymph glands of two cases of rat-bite fever state that they "can be detected in about 3 % of house rats in Japan." They describe the spirochaete which they have named *S. morsus muris*, as having a short thick body "somewhat larger than *S. pallida* but smaller than *S. duttoni* and *obermeiri*. It is thicker than *S. pallida* and measures 1·5 to 2μ to 5 to 6μ," generally thick and short of 2 to 5μ with a flagellum at one or both ends, with $1\frac{1}{2}$ to 6 curves. They state that the flagella may or may not be stained with Giemsa, but Burri's Indian ink method shows them well.

The spirochaetes found by me in rat 84 correspond very closely with their description, and although I cannot see distinct flagella (in Giemsa stained preparations) in most, in a few they are indistinctly made out.

I would express here my indebtedness to Mr Jos. Cooper-Dean, and Mr T. B. Goodall, F.R.C.V.S. for their kindness in procuring specimens from the country and for their interest in the work.

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DESCRIPTION OF PLATE I.

GROUP A.

Figs. 1–4. Spirochaeta Icterohaemorrhagiae from blood of guinea-pig inoculated with blood from a case of Weil s Disease.

Figs. 5–12. Spirochaetes from the emulsion of kidneys of naturally infected rats. Stained Giemsa. × 2000 diameters precisely.

GROUP B.

Figs. 1–3. Spirochaetes from blood of guinea-pig.

Figs. 4–6. Spirochaetes from the kidney of rats. Stained Giemsa. × 3000 diameters precisely.

GROUP C.

Figs. 2 and 3. Spirochaetes from blood of guinea-pig.

Figs. 5–8. Spirochaetes from the kidney of rats. Photographed at × 2000: enlarged twice = × 4000 diameters.

THE BIOLOGY OF *AMBLYOMMA DISSIMILE* KOCH.

WITH AN ACCOUNT OF ITS POWER OF REPRODUCING PARTHENOGENETICALLY[1].

By G. E. BODKIN, B.A., Dip. Agric. (Cantab.), F.Z.S., F.E.S.,
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(With Plate II and 1 Text-figure.)

INTRODUCTION.

Amblyomma dissimile Koch is well known to science and previous accounts of its biology have already appeared. Newstead (1909) made some observations on its habits in Jamaica. Hooker, Bishopp, and Wood (1912) give an exact account of its life cycle worked out in confinement in the Southern United States of America. These authors do not refer to the occurrence of parthenogenesis in this species.

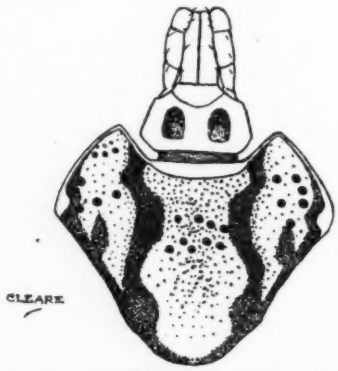

Fig. 1. *Amblyomma dissimile* Koch, ♀, capitulum and scutum. × 15.

Parthenogenesis has not hitherto been recorded in *A. dissimile*. No previous work has been done on the life histories of ticks in British Guiana.

Parthenogenesis has been recorded by Aragão (1912) in Brazil in *Amblyomma agamum* Aragão. In this instance the male of the species has never been seen. In British Guiana, as will presently be described, the males of

[1] Published by permission of the Director of Science and Agriculture, B. Guiana.

A. dissimile, are frequently met with and are capable of performing their functions. It is worthy of note that Newstead failed to capture males of this species in Jamaica.

Nuttall (1915), working in England, has induced parthenogenesis experimentally in *Rhipicephalus bursa*.

In British Guiana *A. dissimile* is well known. The engorged females attached to the head of *Bufo marinus* (bull-frogs, termed locally "crapauds") immediately between the eyes are often seen and are most conspicuous objects.

A. dissimile can only be said to be of indirect and of very small economic importance. Thus the attachment of these ticks to the toads occasionally brings about the death of the host; the toads, which are very numerous, are great insect eaters and perform excellent work in the consumption of many insect pests.

The hosts of this tick are toads, iguanas, and lizards, and they have been also observed on several species of snakes. It is probable that the adult females are capable of engorging on most cold blooded animals; they are incapable of engorging on warm blooded animals. The larvae and nymphs however will thus engorge.

I propose to give firstly an account of my methods of rearing this tick, then a detailed account of its life history and habits, and finally shall describe its powers of reproducing parthenogenetically.

METHODS OF REARING.

Throughout these experiments the host utilized was *Bufo marinus*. The experiments were commenced on the 20th October 1915 and terminated in October 1917. The work was carried on somewhat intermittently owing to the frequent pressure of other work. I have to acknowledge occasional assistance from Mr L. D. Cleare, Jnr., F.E.S.

The illustrations will give a good idea of the kind of cage which was found most convenient for confining the toads and for manipulating the ticks with which they were infested. Shallow, circular glass dishes of uniform size were obtained and between them a cylinder of brass wire net with 20 meshes to the inch and about 6 inches in height was introduced, the dishes closing both ends of the wire cylinder like caps. The diameter of the cylinder was about 10 inches. This arrangement was finally arrived at after numerous trials and was found to answer most satisfactorily. It is sufficiently rigid, effectually confines the crapaud and admits a maximum quantity of air and light. A lead weight was necessary on the top cover to prevent the crapaud jumping up and knocking it off; an event very likely to occur with an energetic crapaud desirous of escaping.

The bottom cover of the cylinder was stood in another shallow, circular and larger, glass dish. In this outer dish water and kerosene were placed; this prevented ticks from escaping or entering the apparatus from the outside.

The table on which these cages rested was insulated by keeping its legs immersed in receptacles containing kerosene and water.

It was found impossible to persuade the captive toads to feed during their confinement. Although frequently offered choice delicacies they preferred slow starvation. Curiously enough in several instances fully engorged female ticks that had dropped from the crapaud were devoured.

The toads were regularly supplied with fresh water and the cages thoroughly washed out every two days. When the engorging ticks were expected to drop off this water was reduced to a minimum to prevent the ticks being drowned. Without water the death of the toad ensued within a week, slightly longer during damp weather. A toad, although apparently dead through the failure of its water supply, speedily revived on adding water, of which they were seen to drink large quantities.

Another remarkable habit of these toads, when in confinement, is the frequent shedding of their skins. On a number of occasions I observed the toads deliberately eating their cast skin. The skin as it peeled off was gradually swallowed by a series of convulsive gulps. This shedding of the skin does not affect the attached ticks.

Few of the toads were confined for less than $1\frac{1}{2}$ months; often they were kept well over two months. Their imprisonment had little effect beyond producing emaciation, and, when released, they made off actively. When first captured and introduced into the cages frantic struggles for escape took place. It was a difficult matter to infest them with ticks for the first week of captivity —afterwards they became docile and were easily handled; in fact some actually appeared to enjoy being handled and would close their eyes and remain perfectly still. A few never became tractable. On the whole they proved admirable subjects for such experiments.

The tick larvae and nymphs were transferred from the tubes to the toads by means of fine camels-hair brushes. This was not found necessary with the adults, where small, smooth pointed forceps were used. Engorged ticks which had dropped were collected every morning from the bottom of the cage. The engorged larvae and nymphs often climbed some distance up the wire netting and were found in the interstices.

The larvae and nymphs attached themselves within a few hours of being placed on the toad. The adults often took a whole day or night before becoming attached satisfactorily. The usual place for attachment was on the back or on the head. The reason why these ticks are so frequently found on the head immediately between the eyes under natural conditions is that the hosts often lie submerged for long periods of time with only the head just showing above water.

The female toads, which possess a smoother skin than the males, were found to be more satisfactory than males for tick infestation and attachment. Females of a large size and healthy appearance were utilized. Nearly all used were procured from the Demerara Golf Course at Turkeyn, East Coast,

Demerara, a few miles out of Georgetown. They are abundant but it is necessary to know their lurking places. In extremely dry weather and in heavy rainy weather they are sometimes hard to find. Their favourite retreats are beneath large rainwater vats, in small swamps containing a thick growth of "mucca-mucca" (*Montrichardia aculeata*), inside old galvanised iron pipes 5" diameter lying in a damp and shady situation, and in holes just above the water level of trenches overhung with grass, in fact any place that is cool, close to water, damp, and well concealed.

In rainy weather there is no more familiar nocturnal sound than the regular, resonant, and guttural vibrations of these creatures which mimic the distant beat of a well tuned motor cycle engine running at a fair speed. It is a most cheery sound though apt to become monotonous.

I have dealt at some length on the interesting habits of these toads both in confinement and under natural conditions because they have a direct bearing on those of their parasites.

As soon as the ticks were replete and had dropped they were transferred to glass tubes, open at both ends and with one end implanted in damp sand and the other plugged with cotton-wool after the method devised by Bishopp (1912). This method acted perfectly. The glass vessel containing the damp sand was usually covered with a wire cage to prevent possible disturbance by rats. In these tubes oviposition and ecdysis took place.

Larvae and nymphs, when moulting, often took up a position directly beneath the plug of cotton-wool on the side of the glass. Occasionally they buried themselves in the sand. The adults, before oviposition, completely buried themselves in the sand; it took them several days to thus dig themselves in. The larvae on emergence from the egg immediately made their way to the top of the tube.

LIFE HISTORY.

The original female ticks (three in number) procured for these experiments were obtained from several toads which had fallen into a large metal tank sunk in the earth and partially filled with water. They had obviously been imprisoned there for some time. The ticks were removed from the toads and brought to the laboratory, where they were placed in tubes with damp sand, and allowed to oviposit.

Oviposition commenced 22. x. 15 and proceeded regularly for about 16 days. On 18. xi. 15 the females were dead. The eggs are light brown, ellipsoidal in shape, smooth and shining. As development of the embryo proceeds dull white markings may be observed. The larvae are active but feign death on being violently disturbed. After emergence they void a tiny white spot of excrement. The larvae await their host with the front pair of legs extended above and in front of their bodies. This position is assumed if they are at rest and then gently disturbed.

The following records do not relate to single ticks but to lots of six or more raised at one time:

Engorgement of larvae (*average temperature* 82·0° F.).

Larvae placed on crapaud	1. xii. 15	10. xii. 15	average period 14 days.
Dropped engorged	17. xii. 15	22. xii. 15	

Engorgement of larvae (*average temperature* 80·1° F.).

Larvae placed on crapaud	22. vi. 17	12. vi. 17	12. vi. 17	5. vi. 17	22. v. 17	average period 15·2 days
Dropped engorged	1. vii. 17	29. vi. 17	23. vi. 17	14. vi. 17	3. vi. 17	

Moulting of larvae (*average temperature* 82·0° F.).

Dropped engorged	23. xii. 15	30. xii. 15	7. i. 16	21. i. 16	average period 15 days.
Ecdysis complete	7. i. 16	13. i. 16	19. i. 16	2. ii. 16	

Longevity of larvae (*average temperature* 82·0° F.).

Isolated	23. xi. 15	52 days. The greatest longevity observed was 84 days*.
Died	14 ii. 16	

Engorgement of nymphs (*average temperature* 82·0° F.)

Placed on crapaud	8. i. 16	8 i. 16	14. ii. 16	10. iii. 16	average period 15·3 days.
Dropped engorged	21. i. 16	24. i. 16	2. iii. 16	27. iii. 16	

Engorgement of nymphs (*average temperature* 80·1° F.).

Placed on crapaud	27. vii. 17	23. vii. 17	4. vii. 17	10. vii. 17	18. vii. 17	average period 11·3 days.
Dropped engorged	4. viii. 17	1. viii. 17	18. vii. 17	24. vii. 17	1. viii. 17	

Moulting of nymphs (*average temperature* 82·0° F.).

Dropped engorged	24. i. 16	3. iii. 16	13. iii. 16	average 15 days.
Ecdysis complete	9. ii. 16	18. iii. 16	27. iii. 16	

Nymphal longevity (*average temperature* 82·0° F.).

Isolated in tube	9. ii. 16	10. ii. 16	average 99·1 days.
Died	18. v. 16	18. v. 16	

Engorgement of adults (*average temperature* 82·0° F.). (Unfertilized females.)

Placed on crapaud	14. ii. 16	20. iii. 16	average 31·1 days.
Dropped engorged	19. iii. 16	18. iv. 16	

Engorgement of adults (*average temperature* 80·1° F.). (Unfertilized and fertilized females. The fertilized ticks took slightly longer.)

Placed on crapaud	26. iii. 17	26. iii. 17†	26. ii. 17	26. ii. 17	26. ii. 17†	average 51 days
Dropped engorged	3. v. 17	5. v. 17	11. iv. 17	4. iv. 17	12. iv. 17	

Adult longevity (*average temperature* 80·1° F.). (Two unfertilized females.)

	♀ A	♀ B	
Isolated in tube	16. iv. 16	16. iv. 16	average 461·1 days
Died	17. vi. 17	25. viii. 17	

* The larvae were kept in glass tubes stuck in damp sand as previously described.

† Fertilized.

Female tick B lived 496 days. On 11. vii. 17 she was placed on a toad (without being fertilized by a male) and soon became attached. When about $\frac{3}{4}$ engorged she dropped off (9. viii. 17) and was placed in a glass tube stood in damp sand. Oviposition took place and the eggs ultimately hatched. This tick therefore lived for 451 days without food and at the end of that period was still able to attach herself, become engorged, and afterwards lay fertile eggs without being attended by a male; truly a most remarkable performance.

The total life cycle of *A. dissimile*, provided that each stage promptly finds a host, takes roughly 153 days as follows:

From the deposition of the eggs to hatching thereof	27 days
Five days allowed for larvae to harden before attachment to host	5 „
Engorgement	15 „
Ecdysis	15 „
One week allowed for nymph to harden	7 „
Engorgement	13 „
Ecdysis	15 „
Ten days allowed for adult to harden	10 „
Engorgement	41 „
From engorgement to production of eggs roughly	5 „
	153 days

Under natural conditions however this period must be very greatly lengthened as it is seldom that a host is found at once.

I have observed copulation repeatedly in the laboratory. A number of males were collected from toads, brought to the laboratory and there placed in tubes with females. In most cases the sexual act took place at once but if the males had been kept in captivity for some time it became a difficult matter to induce them to copulate. The act was observed when females which were about $\frac{1}{4}$ engorged and had to be forcibly removed from their host were placed together with males.

The male approaches the female and after a short examination makes his way beneath her body. Having gained the required position, with his ventral surface in apposition to that of the female, he secures his position by clinging with his legs to the basal joints of the female's legs. His front pair of legs being in advance of the base of the same pair of legs of the female. The other legs, in succession, in similar positions. His hold is most tenacious. The female usually remains more or less quiescent during the whole operation. As soon as the male has attained the requisite position he at once commences to feel with his proboscis for the genital aperture of the female into which he eventually thrusts his mouthparts, with the exception of the palps. A probing motion is set up and soon the mouthparts penetrate deeper. A small quantity of liquid is now observed about the edges of the sexual orifice of the female. This gradually hardens. The female aperture now appears to become gradually enlarged and depressed owing to the continued probing movements of the male. The movements of the male last for a varying length of time; they may even take as long as ten minutes. At last the male slightly lifts

his body and the spermatophore issues from his genital aperture. If the male is forcibly removed at this period he has considerable difficulty in moving off owing to the adhesive quality of the freshly formed spermatophore. As the male raises his body the mouthparts are withdrawn. The spermatophore is now deposited in the slight depression containing the female sexual orifice. The palps and mouthparts of the male are utilized to guide the neck of the spermatophore into the female orifice. The proboscis of the male is now once more inserted and remains thus for about three minutes. Gentle probing movements take place. The male then retires. The spermatophore is now situated with its neck within the sexual orifice of the female. It is rounded in shape and the male was not observed to force out its contents before leaving the female. After about ten minutes it becomes flattened and emptied. The empty capsule remains firmly attached for some time[1].

In nature males and females are found together on the same host though I have never observed copulation to take place there.

Proportion of the sexes. The examination of 30 tick-infested toads yielded 55 adults: 46♂, 9 ♀. Therefore, under natural conditions, the males largely predominate.

The following weights of various stages of *A. dissimile* are of interest. Fresh specimens were used.

Unengorged adult female	·0110 grm.
Engorged nymph	·0270
Engorged larva	·0010

An average sized engorged female measures:

Length	17·5 mm.
Breadth	11·5
Depth	8·0

Parthenogenesis. I have succeeded in definitely establishing that parthenogenesis occurs in *Amblyomma dissimile.*

Two adult female ticks which had been carefully bred from ova in the laboratory and kept in strict captivity in a glass tube plugged with cotton-wool over sand were allowed to engorge on a toad. During engorgement all possibility of a male gaining access to them was rendered impossible by standing the cage in a large dish filled with kerosene and water. The table on which these cages were kept was stood in dishes containing the same mixture. If males were brought in during this same period and kept on the table they were carefully isolated and eventually killed. There was no remote chance of them gaining access to the females.

The two females eventually engorged normally and laid a normal quantity of eggs, all of which, as far as I could ascertain, were fertile.

[1] This agrees with the description of the process of copulation described by Nuttall in *O. moubata, Parasitology,* IV. 39; *Ixodes putus, Ibid.* VI. 81; *Dermacentor venustus, Ibid.* VII. 429; and since observed by him in *Rhipicephalus* spp. and *Amblyomma variegatum.*

It was impossible, owing to the lack of space and equipment, to raise even a quarter of the larvae produced from the eggs laid by one female.

Some of the progeny of these ticks were carefully raised and 65 adult females were eventually secured. Some of these were again allowed to engorge and produce another generation. I have thus raised, from the two original females, four generations parthenogenetically. No diminution either in size or vigour was noticed.

From these results it appears that a generation produced parthenogenetically consists entirely of females.

An attempt was made to determine the results of fertilization.

For this purpose two female ticks (A and B) were selected which for three generations back had been bred parthenogenetically. Males were secured from the field and copulation was allowed to take place. Fertilization took place inasmuch as a spermatophore was in each instance observed to be attached to the sexual orifice of both females.

Of the progeny of ♀ A, owing to several misadventures, only 6 reached maturity; all of these were females. Of ♀ B's progeny 15 reached maturity; one was a male and the others females.

It seems likely that a parthenogenetic generation consists entirely of females; otherwise a small percentage of males results. Hooker, Bishopp, and Wood (1912) obtained a percentage of 71 females in a generation. In this instance it is uncertain, however, that fertilization took place.

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EXPLANATION OF PLATE II.

(Photographs by G. E. Bodkin.)

Fig. 1. *Amblyomma dissimile*, 3♂♂, infesting head of *Bufo marinus*.

Fig. 2. *Amblyomma dissimile*, engorged ♀, in characteristic position on head of *B. marinus*. Slightly under life size.

Fig. 3. *Amblyomma dissimile*, engorged ♀, attached to side of body of *B. marinus*. Slightly over life size.

Fig. 4. Cage used for confining the toads used for raising the ticks. The large dish contains kerosene and water. The lead weight holds the lid firmly in place. For description, see text.

Fig. 5. Tubes used for confining the ticks under experiment. They are planted in damp sand and cotton-plugged above. The shallow dish contains kerosene and water.

Fig. 6. *Amblyomma dissimile*, ♀; ovipositing. About natural size.

LERNAEOPODA SCYLLICOLA N. SP., A PARASITIC COPEPOD OF *SCYLLIUM CANICULA*.

By W. HAROLD LEIGH-SHARPE, B.Sc. (Lond.).

Part II.

(With 11 Text-figures.)

(*The figures are all drawn from specimens mounted in Farrant's medium.*)

Part I of this paper, which dealt exclusively with the females, appeared in *Parasitology*, viii. 262. After examining some dozens of dogfish each month for the previous three years I, at length, on April 2nd 1918 obtained from two separate dogfish caught during the preceding week at Plymouth, three females of *Lernaeopoda scyllicola* which were accompanied by males.

Males, which are rarely collected in the family Lernaeopodidae, are unknown hitherto in some species, and for the genus *Lernaeopoda* have been described only very inadequately as to the appendages in *L. bidiscalis* by Kane (1892), and superficially in two other species, viz. *L. elongata* and *L. galei*. A detailed description of this species therefore supplies a pressing want.

Sexual dimorphism attains striking proportions in this family, the males showing a marked dissimilarity in size, structure of the body, and its appendages, as also in the methods of prehension and locomotion. For, as has been said, though the free-swimming period of the male larva is no longer than that of the female, and the two often fasten themselves side by side upon the same host fish, whereas the attachment in the case of the female is permanent throughout life, it is but temporary in the male. When each partner has arrived at sexual maturity, the male, which is fixed only by the frontal filament, describes, with the point of attachment as centre, an arc until, on coming in contact with the body of the female, he abandons the filament and fastens himself to the female by the second maxillae and maxillipedes, remaining there for the remainder of his life. Not being permanently attached he is able to crawl about over the female's body, but such motion is slow and laborious, and probably performed only when necessary. He does not increase his size at each successive moult like the female, but remains dwarfed showing less degeneration, and more distinct segmentation in the trunk region.

A curious similarity in position is exhibited by each of the attached males. Each was located on the *left* side of the female, immediately behind the cephalothorax. I have previously remarked and figured that the female

shows faint indications of segmentation at the anterior end of the trunk. The first somite by means of a lateral protuberance forms a point of attachment for the male, and will be referred to as the "shoulder," the male's second maxillae being anterior to the protuberance, the maxillipedes posterior to it (Fig. 1).

Although it is generally agreed that a single pair of spermatophores contain sufficient spermatozoa to fertilize all the eggs a female can lay during

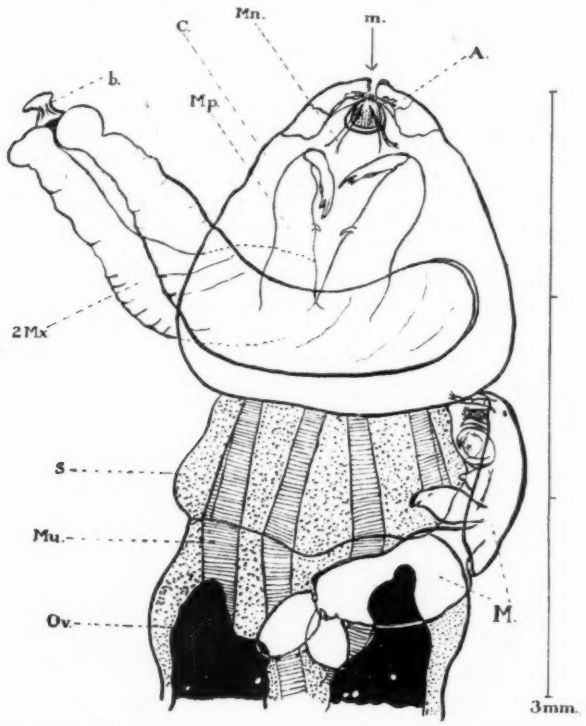

Fig. 1. *Lernaeopoda scyllicola*, the anterior two-thirds of a sexually mature, but not fully-grown female, in ventral aspect, with an attached male (*M*) *in situ*. *b.* bulla; *A.* antennae; *m.* mouth; *Mn.* mandibles; *2Mx.* second maxillae; *Mp.* maxillipedes; *Ov.* ovary; *S.* shoulder; *Mu.* muscles. (The appendages of the female are only roughly indicated; for details see Part I. Fig. 5.)

her lifetime, yet the male may remain clinging to her shoulder for a short period after mating (which takes place upon reaching maturity immediately after the moult at the close of the second copepod stage), since all my specimens of females were provided with well developed, normal egg-strings. It is unlikely, however that the male lives very long after fastening the first pair of spermatophores in place in the spermathecae of the female, judging from rarity of males in this and other species.

I may here record that the dogfish consigned to me from Plymouth during the year 1917, bore remarkably few examples of *Lernaeopoda scyllicola* ♀. In no case were more than two present, and about half the total number was devoid of them. As far as the present year has gone (April, 1918), the number of parasites present appears to show a slight increase. Dividing the number of male dogfish examined into the total number of parasites taken yields the following "periodicity table" of females:

1914 (the latter six months)	**4**
1915	**4** (maximum 6)
1916	**3**
1917	**1**
1918 (the first three months)	**2**

Only two individuals of *L. scyllicola* ♀ were observed on *female* dogfish during the whole of the foregoing period, and those during 1915. Besides the usual locations within the extra-cloacal aperture, and on the grooves of the claspers, I have recently found *L. scyllicola* ♀ in other, probably fortuitous, situations. In April 1918 I took a specimen outside the extra-cloacal aperture posterior to and between the fusion of the pelvic fins and the body, and one specimen (sexually immature) from the extreme tip of the right clasper. Although specimens from the clasper grooves are not uncommon, it is an extraordinary coincidence that hitherto I have only taken them from the dogfish's *right* clasper, and never from the left. I have heard it authoritatively stated by mariners and sea-faring men that sharks and other Elasmobranch fishes at the natatory "roll," or when they turn over to seize their prey, invariably depress the right side of the body, and I have observed the same phenomenon in the rolling of porpoises. Possibly in the rolling dogfish the left clasper is elevated, and the Copepod larvae sucked against the right clasper. I believe that the cloaca of the female dogfish would not be opened at the natatory roll, the pelvic fins having a different conformation posteriorly from those of the male, which may account for the rarity of *L. scyllicola* upon female dogfish.

From recent observations I conclude that the length of the "arms" (2nd maxillae) is so liable to variation, in the genus *Lernaeopoda* at any rate, that no reliance can be placed upon it as a specific character. The "arms" of female specimens taken on the clasper grooves are invariably much shorter than of those attached within the extra-cloacal space, while the one attached to the tip of the clasper had "arms" barely longer than the cephalothorax. Conversely the more anteriorly are the parasites located, the longer do their "arms" become, those situated practically within the cloaca and around the urino-genital papilla possessing the longest "arms" of all. It is obvious that these admit of, and possibly require, more movement about a fixed centre (the bulla), while such radial movement would be disadvantageous to an individual affixed to a clasper, as it would be liable to accidents causing detachment. The other, shorter-armed genera of the Lernaeopodidae are found upon the gills of various fish, where there is little freedom for movement, so that, in fine, the length of the "arms" has a biological rather than a specific significance.

DESCRIPTION OF THE MALE.

Body. The outline of the animal is best seen from Figs. 2, 3 and 4. The animals are of a yellowish white colour, though, as a rule, paler than the female. Length 2 mm. or about one third the length of the female which, though sexually mature, has not yet attained her full growth. The body is

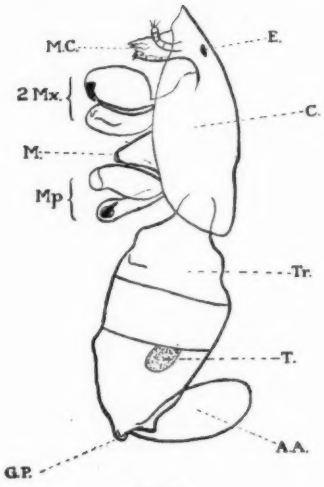

Fig. 2

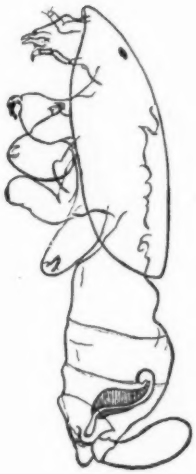

Fig. 3

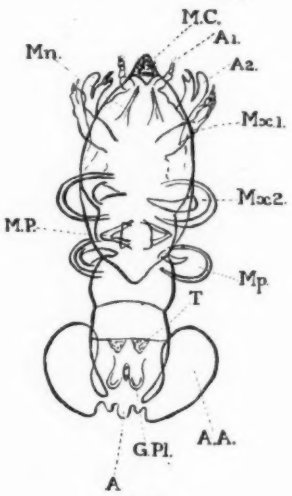

Fig. 4

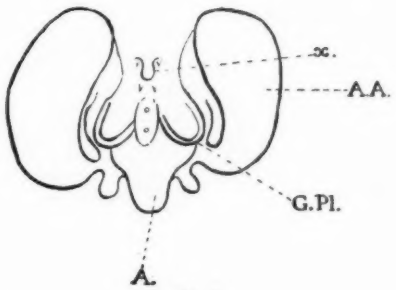

Fig. 5

Fig. 2. *Lernaeopoda scyllicola* ♂, viewed from the left side. *C.* cephalothorax; *Tr.* trunk; *T.* testis; *M.C.* mouth cone; 2*Mx.* second maxillae; *Mp.* maxillipedes; *M.* mediative processes; *A.A.* abdominal appendages. *G.P.* genital process; *E.* eye. (Diagrammatic.)

Fig. 3. *Lernaeopoda scyllicola* ♂, the same as Fig. 2, but in greater detail; the reproductive system is indicated.

Fig. 4. *Lernaeopoda scyllicola* ♂, in ventral aspect. *M.C.* mouth cone; *A*1. antennules; *A*2. antennae; *Mx*1. first maxillae; *Mx*2. second maxillae; *Mp.* maxillipedes; *M.P.* mediative processes; *T.* testes; *A.A.* abdominal appendages; *A.* abdomen; *G.Pl.* genital plates; *Mn.* mandibles. (The anterior appendages are slightly exaggerated.)

Fig. 5. *Lernaeopoda scyllicola.* The posterior end of Fig. 4 enlarged. *A.* abdomen; *A.A.* abdominal appendages; *G.Pl.* genital plates; *x.* genital process (see p. 25).

bent so that the *cephalothorax*, which is proportionately larger than in the female, is more or less inclined to the trunk, from which it is separated by a well-defined groove, covered by a distinct dorsal carapace and strongly flattened dorsi-ventrally. The carapace has a two pointed rostrum. The *trunk* is conical and inflated, and shows traces of segmentation. Three regions are readily distinguishable. There are large paired *anal laminae*, which are curved in such a way as to turn forwards dorsally. These are homologous with the *abdominal appendages* of the female and should be called such. A pair of peculiar processes, found also in other species of *Lernaeopoda* (and perhaps homologous with an unpaired rounded protuberance *between* the maxillipedes in *Brachiella* and *Clavella*) protrude from the body wall between the second maxillae and the maxillipedes. These Kane (1892) calls "Intermaxilliped processes," since he considers the second maxillae to be a first pair of maxillipedes, a view held likewise by other authors, but, as it has been established by Hansen (1893), Giesbrecht (1893), Claus (1895) and Wilson (1910), that the penultimate pair of appendages arises in early development *in front* of the suture which separates the head from the thorax, while the ultimate pair arises *behind* that suture, the former are to be regarded as a second pair of maxillae, and Kane's name is no longer appropriate.

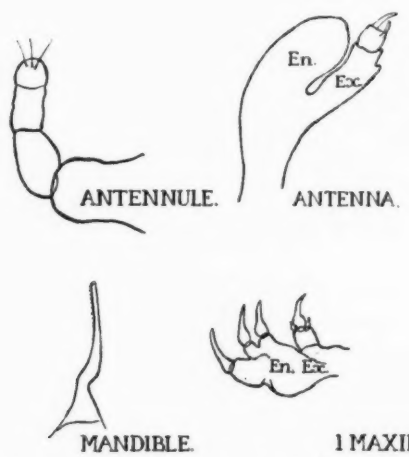

Fig. 6. *Lernaeopoda scyllicola*. The antennule, the antenna, the mandible and the first maxilla. *En.* endopodite; *Ex.* exopodite.

These *mediative* or middle-acting processes, though not prehensile, overlap the female, and may subserve the function of location, fitting, as their pointed ends do, over the protuberance that constitutes the female's shoulder. They shrink very considerably on mounting, and appear glandular rather than muscular.

There is no large postero-ventral spine on the abdomen, but instead a rounded protuberance and two pairs of small spines (Fig. 10).

The Appendages, all paired, are: 1st antennae (antennules), 2nd antennae, mandibles, 1st maxillae, 2nd maxillae, and one pair of maxillipedes, the same members as are represented in the female.

The 1*st Antenna* or *Antennule* (Fig. 6) is four-jointed. The basal joint is the largest, but has no spiral turn as it has in the female. The other joints diminish regularly in size, and the terminal joint is tipped with (two or usually) three sensitive setae.

The 2*nd Antenna* (Fig. 6) consists of a basal joint bearing a large unjointed endopodite with a round or blunted end, and a small exopodite whose diameter is half that of the endopodite, and which is distinctly two jointed, the terminal joint being a claw which forms a true chela in conjunction with a spine projecting from the penultimate joint.

The *Mandibles* (Fig. 6) precisely resemble those of the female. The teeth appear to be vestigial.

The 1*st Maxilla* (Fig. 6) consists of an unsegmented endopodite, and an exopodite or palp which is two-jointed and tipped with a large mammillated claw and two small subsidiary claws. The endopodite is tripartite at the distal end, the outer ramus being terminal, and the other two on the inner margin. Each ramus ends in a long mammillated claw.

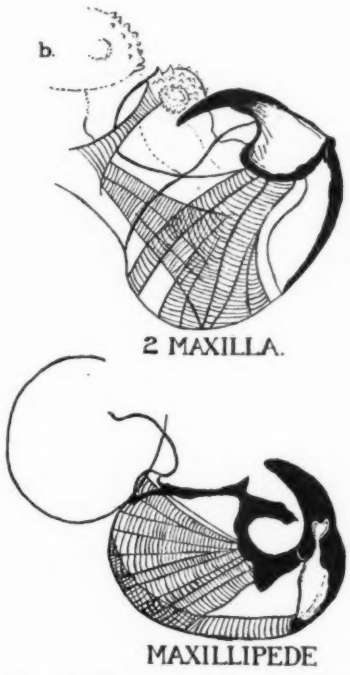

Fig. 7. *Lernaeopoda scyllicola.* The second maxilla and the maxillipede *b.* the pad of spines on the basal joint of the fellow maxilla seen in perspective.

The 2*nd Maxilla* (Fig. 7) is stout, two-jointed, with a large spinous process on the basal joint for the apposition of the terminal claw.

The *Maxillipedes* (Fig. 7) closely resemble the 2nd maxillae. They are two-jointed, and again the terminal claw is curved and fits against a process on the basal joint which is not spiniferous. They are not appreciably larger than the 2nd maxillae, as in most species. They arise close to the bases of these maxillae and are swollen and fleshy, terminating in an auricular disc. The two discs approximate at the inner edge, and thence conjointly give rise to a saucer-like *tenaculum*.

The musculature of the appendages is seen from Fig. 8, and calls for no comment. It is much more powerfully developed than in the female.

The unpaired median nauplius **eye** is retained in these males. Kane also found it in *L. bidiscalis*; but none of the male Lernaeopods described by Wilson (1915) are figured as possessing such an organ. A very noticeable optic nerve connects it with the supra-oesophageal ganglion. The eye appears pink.

The Mouth parts. The mouth forms a suctorial proboscis situated at the summit of a cone which is bent at right angles to the cephalothorax, so as to be directed downwards. There is an upper and a lower lip each of which is bordered by setae of characteristic shape. In appearance it exactly resembles that of the female *q.v.*

The Alimentary canal, like that of many parasites, is degenerate and has no visible contents. A moderately long oesophagus leads into a dilated stomach which tapers off to form an intestine which apparently ends blindly (Fig. 11). Wilson (1915) is emphatic that no male member of the entire family possesses an anus.

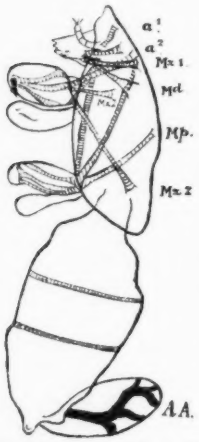

Fig. 8. *Lernaeopoda scyllicola*, showing the musculature of the appendages. a^1. antennular muscles; a^2. antennal muscles; *Mx*1. and *Mx*2. muscles of the first and second maxillae respectively; *Mp.* maxillipedal muscles; *A.A.* chitinous ridges of the abdominal appendages; *Md.* mandible.

No special glands are present apart from those cells lining the canal, so that digestion is probably intra-cellular. Both stomach and intestine are thick-walled, and the inner layer of the latter is stated by Wilson to contain many modified cells which secrete a digestive enzyme, and thus it takes the place of a digestive gland.

The males are merely epiphytic (epizoic) upon the females; certainly not parasitic. It has been suggested either: (1) that the males do not feed at all; hence perhaps the brief duration of their adult life, and the absence of functional teeth on the mandibles, and of an anus (though apparently Kane figures one in *L. bidiscalis*); or (2) that they are commensal, and participate in the meal of cellular tissue which the female has rasped off the unprotected soft portions of the extra-cloacal region of the host. An examination of the fluid extruded from the cloaca of the host under a high magnification revealed large numbers of characteristic spiral-headed spermatozoa of the dogfish, and enormous quantities of the eggs of the parasitic Nematode so well known to infest all parts of the dogfish's alimentary canal. I suggest that he feeds on either of these, either being especially nutrient; if on the former, it may be the failure of the supply of these out of the breeding season (or "temporary season") that leads to his early decease.

The Reproductive System of the male consists of a pair of testes situated in the posterior third of the trunk (Fig. 2). Each testis is a pear-shaped body, obliquely placed, with its pointed end facing dorsally and forwards. The spermatocytes are situated at its base, while spermatozoa in various stages of development appear in the more pointed region. From the pointed end a short curved duct leads in an anterior direction to a relatively large cavity, the *spermatophorogonium*, whose thick and glandular walls secrete the spermatophore. From the spermatophorogonium the *vas deferens* finally descends, parallel at first with the previous duct in a posterior direction, but soon it turns ventrally and passes across the side of, or immediately below the testis, enlarging finally to form the genital aperture of that side. Each genital aperture is bounded laterally by a *genital plate* having a double border,

and is separated from its partner by the *genital process* (*sensu stricto*), homologous with that of the female. Both Kane (1892), and Wilson (1915, Plate XXIX, fig. 22), in which I believe he follows Dana, call these genital plates spermatophores. I do not see how they can possibly be such. They do not appear to me to be solid bodies, nor to contain any contents, but merely spoon-shaped expansions with their concavities facing inwards, towards one another, and, as such, capable of grasping the spermatophores when partially extruded and ready for insertion in the female. Moreover as my females bear egg-strings I presume the eggs are fertilized, which predicates that the spermatophores have already been deposited (Fig. 9).

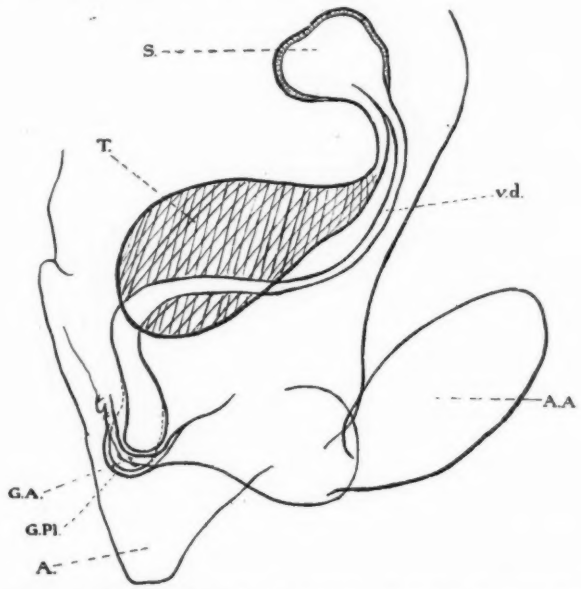

Fig. 9. *Lernaeopoda scyllicola*. ♂ Reproductive system. *T.* testis; *S.* spermatophorogonium; *v.d.* vas deferens; *A.* abdomen; *A.A.* abdominal appendages; *G.A.* genital aperture at the side of the genital process; *G.Pl.* genital plates.

The *genital process* (Fig. 10) bears anteriorly a small rounded projection having a pair of small spines before and a pair behind it, and, posteriorly between the genital plates, two small apertures, one in front of the other, whose significance I have not at present determined.

The Nervous System (Fig. 11) consists of a *supra-oesophageal ganglion* and an *infra-oesophageal ganglion* connected with one another by paired oesophageal commissures which run round either side of the oesophagus.

The supra-oesophageal ganglion, dorsal to the oesophagus, is very noticeable in a preserved specimen not specially stained or treated, situated close behind the eye, which is half the size of the ganglion. The latter is pigmented with a

pinkish-yellow colour. From its anterior end it gives off a readily discernible optic nerve to the eye. From the optic nerve, which is no doubt a compound trunk, branches pass to the antennules, antennae, and possibly to the upper lip.

The *infra-oesophageal ganglion* is large, double the size of the other ganglion, and irregularly shaped. The lower lip and the mandibles are probably innervated from this ganglion, but I have not traced the branches. From the anterior end of the ganglion a pair of nerves arises to supply the 1st maxillae;

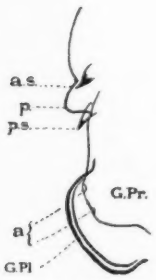

Fig. 10

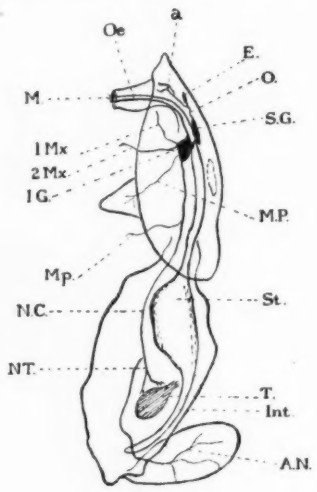

Fig. 11

Fig. 10. *Lernaeopoda scyllicola*, an enlarged view of the genital process (*G.Pr.*) in lateral aspect. *a.s.* anterior spines; *p.* protuberance; *p.s.* posterior spines; *a.* apertures; *G.Pl.* genital plate.

Fig. 11. *Lernaeopoda scyllicola*, the alimentary canal and nervous system. *M.* mouth; *Oe.* oesophagus; *St.* stomach; *Int.* intestine; *T.* testis; *E.* eye; *S.G.* supra-oesophageal ganglion; *I.G.* infra-oesophageal ganglion, a commissure connecting it with the foregoing ganglion; *a.* antennular and antennal nerves; *O.* optic nerve; *1Mx.* nerve to first maxilla; *2Mx.* nerve to second maxilla; *M.P.* nerves to mediative process; *Mp.* nerve to maxillipede; *N.T.* nerves to testis; *A.N.* nerves to abdominal appendages; *N.C.* main nerve cord. (From a preparation treated with osmic acid 1 per cent.)

immediately behind these a pair to the 2nd maxillae. From the postero-ventral surface a pair arises which enters the mediative processes and branches freely within them. From the posterior end springs a main nerve cord which gives off branches to the maxillipedes; these branches (according to other authors) may, however, really arise directly from the ganglion and run coincidently for some distance with the main cord (cf. the origin of the lateral line branch of the 10th nerve in *Scyllium*). The main nerve cord gives off minute branches to the stomach, and a well-marked nerve which bifurcates to the testes. It is continued to the end of the body into the abdominal

appendages in which it ramifies freely, a point seemingly not hitherto made out by other investigators.

Posterior to the supra-oesophageal ganglion and apparently innervated by it, dorsal to the oesophagus, is a body which is probably one of a pair of excretory bodies mentioned by other investigators (the maxillipedal pair).

General note upon the females. From Fig. 1, and by comparing it with Fig. 1, Part I, various discrepancies in size may apparently be detected. The sexually mature females mentioned in this paper, it must be borne in mind, are not fully grown, and they show a much more striking likeness (*inter alia* as to dimensions) to *L. galei*; so much so that I hazard the conjecture that those authors who confounded the two species in former times based their conclusions on the examination of specimens that were not fully adult. Nevertheless the hook on the maxillipede,one of the more prominent characters on which I founded this species, is easily discernible on my young females though it is at present so small as to be more appropriately called a spine. It is obvious from Fig. 1 that the "arms" (2nd maxillae) must elongate considerably hereafter to become in time as long as the trunk, since they are at present not much longer than the cephalothorax. I have also a female so immature that as yet it shows no indications of ovaries; it is not accompanied by a male, and its arms are shorter still.

The Generic characters of the male are at present indeterminable owing to the comparatively few species that have been found. The following characters may be noted: Size larger in proportion to female than in other genera; cephalothorax slightly inclined to trunk, from which it is separated by a constriction; abdominal appendages exceptionally large, and turned forwards dorsally; mediative processes present and well developed; antennule four-jointed; antenna with a terminal claw and chelate; second maxilla with a large spinous process on the basal joint, forming a chela with the terminal claw; maxillipedes resembling the second maxillae.

Specific characters of the male. Cephalothorax strongly flattened dorsi-ventrally and covered dorsally with a distinct carapace which is prolonged forwards into a two-pointed rostrum. Cephalothorax not much inclined to trunk, which is of about equal length, cylindrical, inflated, and distinguishable into three regions as well as a minute posterior abdomen. Genital process with two pairs of minute spines and two apertures. Trunk without large postero-ventral spine. The abdominal appendages narrow into a slender neck where they join the abdomen, then swell into large bodies rounded at the extremities. Single median eye present. Maxillipedes not appreciably larger (if any) than the 2nd maxillae.

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LERNAEOPODA GLOBOSA N. SP., A PARASITIC COPEPOD OF *SCYLLIUM CANICULA*.

By W. HAROLD LEIGH-SHARPE, B.Sc. (Lond.).

Part I.

(With 3 Text-figures.)

(The figures are all drawn from preparations mounted in Farrant's medium.)

Habitat and Record. On 10. iv. 1918 my attention was called to five specimens of *Lernaeopoda* occurring on *Scyllium canicula* obtained at Plymouth. The largest copepod was attached outside and about one inch ventral to the left spiracle. The other four were deeply concealed within the left nasal groove, so that the nasal flap had to be cut away in order to detach the parasites. These specimens exhibit characters of their own which seem to combine those of other established species. The most striking modification is that the ends of the attaching "arms" (2nd maxillae) are expanded into large discs on either side of a vestigial bulla, thus recalling *L. bidiscalis* Kane, 1892, though that species is described as possessing discs of a different conformation, and as having no bulla. In other respects they agree more with *L. musteli* Thomson, 1899, notably as to their small size, and the proportion of the arms to their trunk. But to these points I do not attach much importance, since both may be due to the confined space in which the parasites are domiciled. Indeed I would not have mentioned them at all but that no recorded *Lernaeopoda* of the well-established species obtains such small dimensions. *L. musteli* Thomson, however, was diagnosed from a single specimen, and has never been met with again. No details of appendages etc. were given, so that the species remains practically a nominal one. Since it was (1) taken from the cloaca, (2) of *Mustelus antarcticus*, (3) near New Zealand, it is most improbable that my specimens should be of that species. I therefore hold that this is a species new to science, and for it I propose the name *Lernaeopoda globosa*[1].

Only the females are dealt with in this paper. A common or central type (*L. scyllicola*) was described by me in 1915, and may be referred to for purposes of comparison.

[1] Later, a single specimen of *L. globosa* was taken from the right nasal groove of *S. canicula*, one of a batch of six landed at Plymouth on May 21st, 1918. The specimen was perfect, and possessed two ovisacs. The new species therefore does not appear to be uncommon.

Body. The outline of the animal is best seen from Fig. 1, which is drawn to scale. The total length of the type specimen from the "top" (dorsal side) of the cephalothorax to the end of the egg-sacs is but 5–6 mm., of which the cephalothorax occupies 1 mm., the trunk a little over 2 mm., and the egg-sacs also a little over 2 mm. The arms are much curved, and, though almost as long as the entire animal, the bulla is inserted but 1 mm. above the "top" of the cephalothorax.

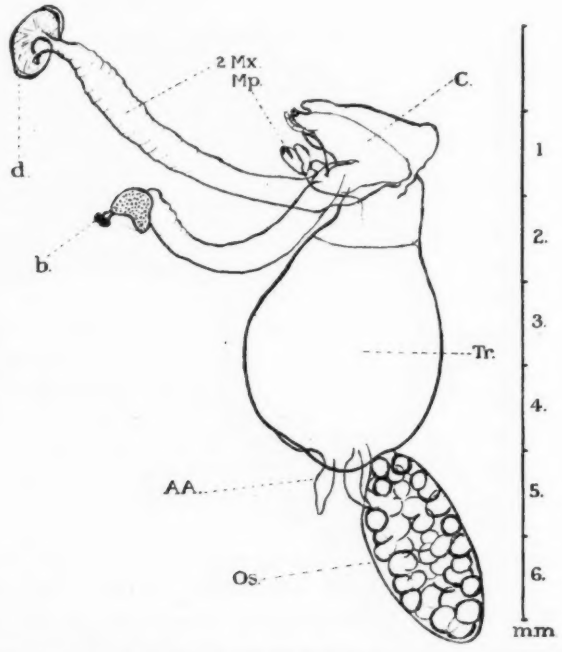

Fig. 1. *Lernaeopoda globosa*, viewed from the left side The "arms" are represented as parted, otherwise the bulla would not show in this position, and one of the discs appears to be damaged. *C.* cephalothorax; *2Mx.* second maxillae; *b.* bulla; *d.* disc; *Mp.* maxillipedes; *Tr.* trunk; *Os.* ovisacs; *A.A.* abdominal appendages.

The abdominal appendages are almost filiform and minute, being but 0·5 mm., or less than a quarter as long as the egg-sacs. They are *ventral to the ovisacs* (generic character), and diverge but slightly from one another.

Th Cephalothorax is comparatively large, *at right angles* to the trunk axis (a feature unique in this genus), flattened but slightly dorsi-ventrally, and covered with a distinct dorsal carapace.

The *Trunk*, far from being slender, is always much inflated, so as to be globose, or pear-shaped. It is but twice the length of the cephalothorax. The greatest rotundity is ventral. This globular appearance of the trunk (taken in conjunction with the bent attitude of the arms, and the short, broad ovisacs)

will render this new species easily recognisable to the unaided eye. The trunk is joined to the cephalothorax by a stout neck, in which an anterior region is demarcated, but there does not appear to be any projecting "shoulder" for the location of the male as in *L. scyllicola*. The animals are of the usual pale, translucent, straw colour, but the ovisacs are densely white and opaque.

The Appendages, all paired, are the first antennae (antennules), second antennae, mandibles, first maxillae, second maxillae and one pair of maxillipedes.

The 1*st Antenna* (*antennule*) (Fig. 2) is four-jointed, the basal joint being much enlarged, somewhat spherical and having a curious spiral turn upon itself. The other joints are of approximately equal size, if anything the middle joint is the smallest. The terminal joint bears three long spines. Except in the last trifling detail, this appendage is the same as in *L. scyllicola*.

The 2*nd Antenna* (Fig. 2) consists of a basal joint bearing a large unjointed endopodite with a blunted end, and a small exopodite whose diameter is a quarter of that of the endopodite, and which is distinctly two-jointed, the terminal joint bearing three small spines. This conforms exactly with the description of *L. galei*, and not with that of *L. scyllicola*.

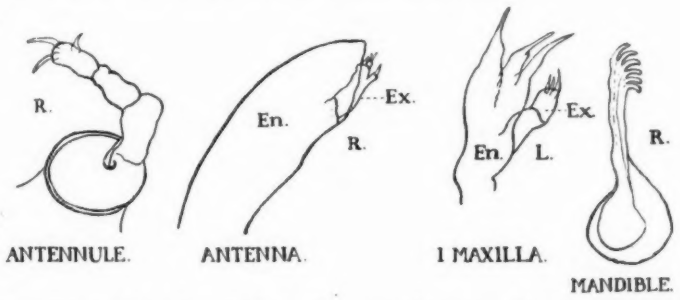

Fig. 2. *Lernaeopoda globosa*, the appendages. The antennule, antenna, first maxilla, and mandible. *Ex.* exopodite; *En.* endopodite.

The *Mandible* (Fig. 2) resembles that of the majority of the Lernaeopodidae, and is distinctly in contrast with that of *L. scyllicola*. The teeth are rounded and point posteriorly on the inner side of the blade; six appears to be a typical number, of which the first two are slightly the larger, the first being terminal; the others decrease in size posteriorly. The extremity of the shaft thus recalls the arrangement met with in certain uncini of post-larval specimens of *Arenicola*, a point I have not sufficiently accentuated in the figure. It would appear, then, that those species which live on the softer tissues (gills, olfactory sacs as in this species) have rounded teeth, while *L. scyllicola*, which has to rasp away the epidermal cells of the extra-cloacal region, or claspers, has serrate teeth.

The 1*st Maxilla* (Fig. 2) is the most constant appendage throughout the genus *Lernaeopoda*. It consists of an unsegmented endopodite, and a well

developed exopodite or palp which is jointed and tipped with three short spines, one of which is somewhat longer than the other two. The endopodite is tripartite at the distal end, one ramus being terminal, and the other two on the inner margin. Each ramus ends in a long slightly mammillated spine, and the conformation recalls the male rather than the female of *L. scyllicola*.

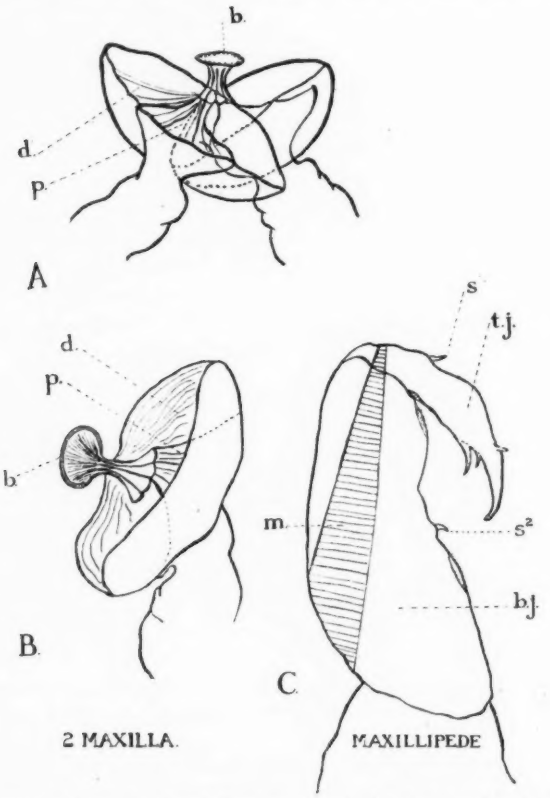

Fig. 3. *Lernaeopoda globosa*, the appendages continued. A. the distal ends of the second maxillae in front view, from a flattened preparation, so that the lower portions of the discs are pressed over one another and appear to overlap. B. the distal end of a second maxilla in side view with bulla half attached. C. the maxillipede. *b.* bulla; *d.* disc; *p.* prominence joining the bulla to the centre of the disc; *b.j.* basal joint; *t.j.* terminal joint; s^1. spine (specific); s^2. spine in same position as hook in *L. scyllicola*; *m.* muscle.

The *2nd Maxillae* (Figs. 1, 3 A and 3 B) are nearly as long as the body, slender, cylindrical, bent sharply half way along their length, and expanded at the distal ends into a large sucker-like organ, the *disc*. They are the organs of attachment to the host. The two are partly in contact with one another, and partly fused to a minute *bulla* or *button*, which is almost vestigial in this species. In detaching the parasites it is very difficult not to leave the bulla

behind. When first they arise in the meta-nauplius stage, these appendages are clawed and similar to the maxillipedes (in other species). In the free-swimming or first copepod stage, when the larva attaches itself to the host, the 2nd maxillae become fastened to the proximal end of the frontal filament. Fig. 3 A is drawn from a flattened preparation, so that the bottom portions of the discs appear to overlap, and in it I have endeavoured to show, that from the centre of the concavity of each disc a prominence arises, which on fusing with its partner from the other disc passes almost imperceptibly into the bulla; thus, in Fig. 3 B (in side view) only half the bulla is shown to be attached. The bulla is embedded beneath the cellular tissues of the host. The inner half of the disc is in contact with the inner half of its partner, while the outer or more distal half overlaps a papilla of the host in which the bulla is embedded, the papilla being induced by the clasping together of the discs. The result is the same as in the bidiscoid condition described by Kane in *L. bidiscalis*, though from his figures the details appear to be different. It is extremely difficult to detach parasites intact from Elasmobranch claspers; invariably the bulla is left behind. Now Kane collected all his specimens from the tips of the claspers of *Galeus vulgaris*. As no other species of *Lernaeopoda* is without a bulla I throw out the suggestion that *L. bidiscalis* may possess a vestigial bulla also, and it is within the bounds of probability that Kane overlooked it.

The *Maxillipedes* (Fig. 3 C) are clawed appendages situated between the bases of the second maxillae, and consist of a basal joint, which is stout instead of being slender as in *L. scyllicola*, and well supplied with powerful muscles which move the terminal joint and flex it down against the inside of the basal joint which is provided on the inner side with two but slightly developed rounded cushions (as compared with *L. scyllicola*), covered with minute spines. Between these cushions is a small spine in the position where *L. scyllicola* bears a large curved hook. The terminal joint, though slender, is not so long and slender as in *L. scyllicola*, and terminates in a long abruptly hooked claw, with two accessory claws at its base on the inner side. On the outer side of the terminal joint there is a small spine at the base of the large claw, and, also, at the proximal end, in an unique position, a further specific spine (Fig. 3 C *s'*). Near the proximal end of the terminal joint on its inner side there is barely recognisable a rounded cushion of small spines, which, when the joints are brought together, would be nearly, but not quite, opposite the more distal cushion on the basal joint. The maxillipedes have lost their function as attaching organs, and are used only for pulling the head down to the host while the animal feeds.

The **Mouth parts,** lips and **Alimentary canal** are as described in *L. scyllicola*.

The **Reproductive System** is in the main as described for *L. scyllicola* save that the oviducts leave the ovaries at the anterior outer borders instead of half way along their sides. The ovisacs, however, are characteristic. Far from

being long and slender, they are short and thick, only as long as the trunk, and end bluntly in a cigar-shaped manner. They contain 6–8 rows of but 12 eggs each. The eggs are comparatively large, spherical, and slightly flattened at the poles. Each of the specimens taken from the nasal grooves (but not the one near the spiracle) bore but one ovisac. This is probably not normal, since both ovaries were fully developed, but due to the confined space in which they were located.

The male dogfish in whose nasal grooves the representatives of this new species were discovered, possesses three specimens of *L. scyllicola*, two of them with attached males, in its extra-cloacal aperture.

Generic characters of female. See *Parasitology*, VIII. 272.

Specific characters of female. Size small (5–6 mm.). Cephalothorax at right angles to the trunk axis. Second maxillae nearly as long as the body, much curved, and expanded at the tips into large cupuliform discs which unite with a minute vestigial bulla. Abdominal appendages almost filiform and minute (0·5 mm.), less than a quarter as long as the ovisacs. Ovisacs short and broad, of the same length as the trunk (a little over 2 mm.). Trunk *globose* or pear-shaped. Second maxillae much bent in a characteristic attitude instead of being raised straight above the head. Mandibles with a few curved teeth. Maxillipedes with a specific spine on the outer edge of the terminal joint, and a small spine on the basal joint, which is stout, where *L. scyllicola* has a large curved hook.

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TRYPANOSOMIASIS OF CAMELS IN RUSSIAN TURKESTAN[1].

By W. L. YAKIMOFF and others.

(With Plates III—V.)

I. INTRODUCTION.

The existence of trypanosomiasis amongst the ruminants of Turkestan has been suspected for many years, but the first definite record is that of Lühs (1906) who discovered *Trypanosoma theileri* in the blood of cattle in Transcaucasia. This was followed by the discovery of the same parasite in the Trans-Baikal Region (Dudukaloff), the Don Province (Stolnikoff) and in the Pskov and Vitebsk Governments (Yakimoff and Bekensky). Wrublewski found this trypanosome in the blood of bison from the Bieloviejsky forests and later it was discovered by Yakimoff in Turkestan.

Feinschmidt (1912) was the first to record the presence of trypanosomiasis amongst the camels of the Astrakhan Government and the following year Yakimoff and his collaborators (1913) confirmed this discovery. Schukewitsch (1914) also observed trypanosomes in the blood of camels from the Ural Region.

From the foregoing records it will be seen that the presence of trypanosomiasis in Russia is far more extensive than had been supposed, and the number of animals suffering from it is gradually increasing.

II. THE DISTRIBUTION OF TRYPANOSOMIASIS OF CAMELS IN TURKESTAN[2].

The proximity of Turkestan to Afghanistan and India, where surra occurs in camels, led us to suppose that camel trypanosomiasis would be most likely to occur in the southern latitudes of our country. Accordingly, enquiries were made amongst the local veterinary surgeons, but with one exception without result. In this case a veterinary surgeon, Skvortzoff, of the Tashkent District, stated that he had heard of the occurrence of a camel disease in the district, called by the natives "Surah," and attributed by them to the animals feeding on a certain kind of plant. We first examined the blood of

[1] From the laboratory of the George Speyerhaus Expedition for the study of tropical diseases in man and animals in Turkestan.

[2] By W. L. Yakimoff, N. T. Shokhor, S. D. Veniaminoff, A. P. Demidoff, A. K. Karpoff, P. A. Novikoff, K. N. Semenoff, Z. A. Ivanoff, T. A. Kolpakoff, S. T. Saikowitsch, and M. F. Korniloff.

the camels in the market of Tashkent and afterwards extended our investigations to other towns and villages in the district, examining the animals at the caravanseries, or in the markets.

Altogether 502 animals were examined, most of them during the months of July and August, and trypanosomes found in the blood of 22, giving a total percentage of infection of 4·38 %. The occurrence according to localities is shown in the following table.

Table I.

Localities	No. of camels examined	No. of cattle infected	% infected
Old Bokhara	104	1	0·95
Tashkent	191	3	1·6
Termezh	90	3	3·33
Koushka	91	15	16·6
Samarkand	26	0	—

From this table it will be seen that the percentage of infection varies widely in different localities, being most common on the Afghan frontier (Koushka and Termezh), and in addition trypanosomiasis has been discovered amongst the camels of Samarkand and Askhabad by inoculating blood into guinea-pigs, although trypanosomes were never actually seen in the camels themselves.

Guinea-pigs were infected with the virus from the following localities, in each case the laboratory animal being injected with blood from a number of camels, until it showed infection.

(1) *Bokhara virus.* During the month of August a guinea-pig was injected subcutaneously with 1–3 drops of blood from each of 230 camels. On the 26th August trypanosomes were observed in its blood and a second guinea-pig infected from it.

(2) *Termezh virus.* On the 10th August a guinea-pig was inoculated with blood from 70 camels; 11 days later trypanosomes appeared in the circulation and a second guinea-pig was infected from it, after an incubation period of five days.

(3) *Samarkand virus.* A guinea-pig was infected by the inoculation of the blood from more than 50 camels. The incubation period in this case was 17 days.

(4) *Askhabad virus.* A guinea-pig was infected by the inoculation of blood from about 70 camels; the incubation period was uncertain. A rat and two guinea-pigs were infected from this animal.

All these four strains of camel trypanosomiasis have been transported from Turkestan to Petrograd.

It has been clearly demonstrated, therefore, that trypanosomiasis of camels is widespread in Turkestan, including the regions of Trans-Caspian Samarkand, Sir Daria and Bokhara; doubtless it also occurs in other regions of that province and it would not be surprising to find it in the southern and

middle parts of European Russia, where camels are imported from Astrakhan and the Urals.

III. MORPHOLOGY OF THE TRYPANOSOME[1].

In fresh preparations the living parasite exhibits fairly active movements, slower than those of *T. brucei* but more active than *T. equiperdum* (Russian strain).

In stained preparations the aspect of the parasites varies somewhat in different experimental animals.

(*a*) *Camels.* Protoplasm slightly spongy; posterior end somewhat rounded. Nucleus oval, well-defined and granular; blepharoplast lightly stained, with a light zone in proximity to it; undulating membrane feebly developed.

(*b*) *White mouse.* Protoplasm spongy; posterior end more rounded. Nucleus granular and containing 2–4 more densely staining particles. Blepharoplast above the light zone. The flagellum of undulating membrane directly enters the blepharoplast.

(*c*) *Rat.* Protoplasm more or less homogeneous; posterior end more pointed. No light zone near blepharoplast; undulating membrane well developed.

(*d*) *Guinea-pig.* Protoplasm more or less uniform; posterior end rather pointed. Undulating membrane well developed; flagellum starting from the blepharoplast.

(*e*) *Rabbit.* Protoplasm not homogeneous. Posterior end rounded.

(*f*) *Dog.* Protoplasm granular; blepharoplast often stains feebly. Involution forms frequently encountered.

(*g*) *Horse.* Protoplasm homogeneous. First day of infection parasites attenuated and slender; later they have the more common shape.

(*h*) *Cattle.* Protoplasm areolar; posterior end rounded. Undulating membrane well developed; flagellum starting directly from blepharoplast.

(*i*) *Sheep.* Protoplasm areolar; posterior end rounded. A light zone near blepharoplast.

(*j*) *Swine.* Protoplasm homogeneous, but granular; posterior end blunter. Chromosomes visible in nucleus; flagellum rather short.

This parasite approximates to the general type of the Nagana and Surra trypanosomes. Multiplication is by longitudinal fission.

IV. THE DIMENSIONS OF THE TRYPANOSOME OF TURKESTAN CAMELS[2].

Since the measurements of trypanosomes are in some cases characteristic of the species of parasite, we have paid considerable attention to this detail, and have measured trypanosomes from camels and also from other experimentally infected animals. Also through the kindness of Messrs Feinschmidt and Shukewitsch we have measured specimens obtained from camels from the Astrakhan and Ural districts.

In all cases the following measurements were taken: total length including flagellum; the length without the flagellum; total width; length of nucleus;

[1] By W. L. Yakimoff.
[2] By W L. Yakimoff and S. K. Popoff.

and length of free part of flagellum; in addition, the distance from the posterior end to the blepharoplast; from the latter to the nucleus; and from the nucleus to the anterior end.

(*a*) Comparison between the dimensions of the Turkestan trypanosome and those from Astrakhan and the Ural region.

The results of the measurements of a number of trypanosomes from camels from these three sources are shown in the two following tables, Table 2 showing the actual measurements and Table 3 the averages (in microns).

Table 2.

Origin of trypanosomes	Total length	Length without flagellum	Width	Length of nucleus	Length of free portion of flagellum
Turkestan	20·94–24·49	13·84–17·75	1·42–2·84	2·13–3·55	4·26–8·52
Astrakhan Govt.	18·81–23·46	11·78–16·84	1·42–2·13	1·77–4·97	3·55–7·81
Ural region	19·52–25·20	15·26–18·10	1·42–2·13	2·13–2·84	4·26–7·10

Table 3.

Origin of trypanosomes	Total length	Length without flagellum	Width	From posterior end to blepharoplast	From blepharoplast to nucleus	Length of nucleus	From nucleus to anterior end	Length of free portion of flagellum
Turkestan (Bokhara)	22·61	15·93	2·13	1·77	5·96	2·76	4·97	6·67
Astrakhan Govt.	19·20	14·3	1·70	2·0	4·5	3·1	4·3	5·9
Ural region	21·97	16·43	1·98	2·41	5·75	2·76	5·11	5·53

From these results it would appear that the Astrakhan camel trypanosome is the smallest, but the differences between the three forms are so small that it is impossible to base any distinction of species on them.

(*b*) Comparison between the dimensions of the Bokhara trypanosomes in various experimental animals.

The results of measurements of trypanosomes taken from the blood of the following animals are given in microns in the following two tables (4 and 5). In every case the animal was infected with the Bokhara strain of camel trypanosome.

Table 4.

Host	Total length	Length without flagellum	Width	Length of nucleus	Length of free portion of flagellum
Mouse	20·94–25·91	14·55–17·39	2·13–2·84	2·13–2·84	4·26–9·94
Rat	18·81–25·91	13·84–20·23	2·13–2·84	2·13–3·55	4·97–8·52
Guinea-pig	26·65–27·33	14·55–20·23	1·42–2·84	2·13–2·84	7·10–9·94
Rabbit	19·52–27·33	14·55–18·81	1·42–2·84	2·13–2·84	4·26–8·52
Dog	20·23–27·69	13·13–19·17	1·42–2·84	2·13–2·84	5·68–8·52
Calf	20·23–24·85	13·84–18·81	1·42–2·84	2·13–4·26	4·26–8·52
Horse	18·81–24·85	13·13–22·37	1·42–2·84	2·13–2·84	2·13–8·52
Pig	18·46–28·75	15·62–21·65	1·42–3·55	2·13–4·26	2·84–9·94

Table 5.

Host	Total length	Length without flagellum	Width	From posterior end to blepharoplast	From blepharoplast to nucleus	Length of nucleus	From nucleus to anterior end	Length of free portion of flagellum
Mouse	23·14	15·76	2·13	2·13	5·96	2·62	4·47	7·38
	23·10	16·43	2·84	2·20	6·03	2·84	4·97	6·67
	23·70	16·46	2·69	1·84	6·03	2·76	5·46	7·24
Rat	22·07	15·89	2·55	1·91	9·24	2·84	4·54	6·17
Guinea-pig	24·84	16·18	2·41	2·20	6·31	2·76	4·54	8·66
Rabbit	22·76	16·11	2·27	2·13	6·03	2·69	4·89	6·49
Dog	22·39	15·01	2·13	2·20	5·11	2·62	4·68	7·68
Calf	22·46	16·22	2·34	1·63	5·60	2·91	5·53	6·24
Horse	22·46	16·64	2·34	1·56	5·82	2·41	6·31	6·82
	21·97	13·87	2·05	1·49	5·18	2·34	4·33	8·09
Pig	23·35	18·17	1·70	2·34	5·75	2·91	6·67	5·18

The results in Table 4 show that the greatest divergence, 18·46 μ and 28·75 μ, occurs in the same animal, the pig.

The average dimensions show very little differences. Even in the same animal, a horse, the average length was 21·97 μ one day and 22·46 μ the next. Excluding the former of these two measurements, the maximum difference in length between the trypanosomes from any two experimental animals is only 2·77 μ, that of the rat being 22·07 μ, and of the guinea-pig 24·84 μ. These measurements are almost identical with those of trypanosomes from the blood of their natural host, and it may be stated that the average dimensions of the trypanosomes from experimental animals compared with each other, and also with those from naturally infected camels, show no appreciable differences.

(*c*) Comparison of the dimensions of the Turkestan camel trypanosome with those of other pathogenic trypanosomes.

The two following tables (6 and 7) give the dimensions, actual and average, of various species of trypanosomes in both mice and guinea-pigs.

Table 6.

	Total length	Length without flagellum	Width	Length of nucleus	Length of free portion of flagellum
In mice					
T. brucei	17·04–25·20	14·20–19·52	2·13–4·26	2·13–3·55	2·84–5·68
equiperdum (Russian)	17·75–24·49	13·13–19·17	2·13–3·55	2·13–2·84	2·84–7·10
,, (French)	18·46–22·72	13·49–17·04	2·84–2·84	1·42–2·84	4·26–5·68
togolense	17·04–23·43	12·78–17·75	2·84–3·55	1·42–2·84	4·26–8·52
gambiense	19·52–22·72	13·84–17·75	1·42–2·84	1·42–2·84	4·26–7·10
hippicum	16·68–23·78	12·42–18·10	2·13–3·55	2·13–4·26	4·26–5·68
In guinea-pigs					
T. evansi	20·94–23·78	14·55–18·10	2·13–2·84	2·84–2·84	4·26–7·10
equinum	18·46–25·56	14·20–19·88	2·13–2·84	2·84–2·84	2·84–8·52
soudanense	18·81–23·78	11·00–18·10	2·13–2·84	1·42–2·84	4·26–8·52

Table 7.

	Total length	Length without flagellum	Width	From posterior end to blepharoplast	From blepharoplast to nucleus	Length of nucleus	From nucleus to anterior end	Length of free portion of flagellum
In mice								
T. brucei	20·62	16·64	3·05	1·98	4·68	2·76	6·67	3·91
equiperdum (Russian)	20·55	15·79	2·84	1·77	4·61	2·41	6·47	4·75
,, (French)	20·05	15·44	2·84	2·13	4·04	2·34	6·24	4·61
togolense	20·44	14·48	2·92	1·42	4·82	2·27	5·25	5·96
gambiense	21·43	15·90	2·43	1·49	5·18	2·41	6·39	5·53
hippicum	20·23	15·33	2·84	1·77	4·89	3·19	5·11	4·89
In guinea-pigs								
T. evansi	22·11	16·57	2·69	1·77	5·75	2·84	5·82	5·53
equinum	22·08	16·54	2·69	7·45		2·84	6·24	5·53
soudanense	20·87	15·12	2·84	1·63	5·25	2·41	5·32	5·75

The two species which especially interested us are *T. evansi* and *T. soudanense*, and from the above figures it will be seen that the total length of the Turkestan parasite exceeds those of the above-named by about 2·73μ, but this difference is insufficient to warrant the establishment of a new species. There is a well-marked difference from the other pathogenic species.

Summarising the results of our investigations on this subject the following conclusions may be stated:

(1) The trypanosomes of camels from Bokhara, the Ural and the Astrakhan regions present no differences in size.

(2) By passage through various experimental animals, both small and large, the trypanosomes from Turkestan camels do not alter in size.

(3) The Turkestan camel trypanosome differs in size somewhat from other pathogenic trypanosomes and even slightly from the geographically related *T. evansi* of India, but measurements alone are insufficient to distinguish the two.

V. VITALITY OF TRYPANOSOMES[1].

The vitality of the trypanosomes *in vitro* was tested at + 17° C. and also at 0° C. Blood containing trypanosomes was drawn from a foal and after being defibrinated was kept at these two temperatures.

(1) *Preservation on ice.* The defibrinated blood was placed on ice on October 9. Four days later mobile trypanosomes were still present. On October 17, none could be found and the inoculation of the blood into a mouse produced no infection.

(2) *Preservation at 17° C.* After being kept for seven days no trace of trypanosomes could be found and inoculation into a mouse also gave negative results.

[1] By W. L. Yakimoff.

VI. AGGLOMERATION OF TRYPANOSOMES[1].

The reaction of the trypanosomes was tested with the following sera:

(1) Normal serum from man, rabbit, horse and bull.

(2) Serum from calf and rabbit infected with this trypanosome.

(3) Horse anti-immune serum against anthrax, epidemic abortion, pig erysipelas, and cattle anti-immune serum against septicaemia and chicken cholera[2].

The results are shown in the following tables.

Number of minutes after mixture of trypanosomes and serum	Observations
	Normal human serum.
1	The trypanosomes begin to unite into rosettes.
3	Well-formed rosettes.
4	Beginning of divergence of the trypanosomes.
8	Their re-union.
9	Divergence.
10 & 12	In some places are seen agglomerated trypanosomes.
20 & 43	Many are separated.
53	All have separated.
	Normal rabbit serum.
1	The trypanosomes rapidly unite into small groups.
3	Numerous small rosettes.
5	Ditto.
8	The number of rosettes greater.
12	Ditto.
13	The rosettes begin to diverge.
14	The trypanosomes begin to diverge rapidly from the rosettes.
16	Nearly all have separated.
	Normal horse serum.
1	Separate trypanosomes slightly mobile.
3	Separate trypanosomes more mobile.
5	Trypanosomes begin to accumulate into small groups.
10	Small groups; no large rosettes.
14	Separate individuals in lively movement.
21 & 24	Ditto.
37	All have separated.
48	Ditto.
	Normal bovine serum.
2	Numerous immobile trypanosomes.
3	Trypanosomes moving slowly and accumulating into small groups.
4	Small rosettes.
6	Numerous small rosettes.
8	In some places the rosettes are larger.
10	All the rosettes begin to part.
13	Many separated rosettes.
23	All the trypanosomes have finally separated.

[1] By W. L. Yakimoff and W. T. Wasilevsky.

[2] These sera were obtained through the kindness of W. T. Routschkin, to whom our thanks are due.

Serum of rabbit infected with the camel trypanosomes.

Number of minutes after mixture of trypanosomes and serum	Observations
1	Trypanosomes immobile.
2	Some exhibit faint movements.
4	Slow agglutination into small rosettes.
5	Numerous small rosettes.
6	Ditto.
7	Accumulation into large rosettes.
9	Many fairly large rosettes.
11	Rosettes moving rapidly.
13	Trypanosomes begin to converge slowly.
14	Many diverging.
18, 32, 34	Ditto.
42, 46 & 66	Ditto.
72	Nearly all separated; immobile rosettes present.
75	Separate individuals slightly mobile.
79	All have separated. The remaining are immobile.

Serum of bull infected with the camel trypanosomes.

1	Trypanosomes agglomerating strongly.
3	Great number of rosettes.
14	Rosettes begin breaking up.
20	Rosettes in some places. Many separate individuals.
36	Only small rosettes with separate individuals remaining.
48	All trypanosomes separated.

From the above described results it will be seen that human serum has the most marked effect on the camel trypanosome and in descending order that of the rabbit, horse and bull. Although bovine serum agglutinates the trypanosomes more rapidly than horse serum, the time during which it retains this power is less.

The action of the serum of animals infected with the camel trypanosome varies in different species. Whilst that of an infected calf acted more energetically than the serum of a normal animal, the serum of an infected rabbit although taking longer to produce agglutination maintains its action for a greater period than that of normal rabbits.

Anti-anthrax horse serum.

Number of minutes after mixture of trypanosomes and serum	Observations
1	Trypanosomes faintly mobile.
2	Trypanosomes more mobile.
3	Accumulation into groups of 3–5 individuals.
5	Small rosettes.
6	Large rosettes.
10	Rosettes diverging, sometimes rapidly, sometimes slowly.
11	In some places small rosettes.

Anti-anthrax horse serum continued.

Number of minutes after mixture of trypanosomes and serum	Observations
28	Rosettes diverging; in some places small groups.
34	Numerous, separated trypanosomes.
53	Diverging rosettes still present.
60	They slowly diverge.
65	Nearly all have parted.
85	All have finally separated.

Serum of horse immune against epidemic abortion.

1	Trypanosomes immobile.
2	Some individuals started moving.
4	Slow agglomeration.
6	The union effected.
8	Small rosettes.
9	Rapid divergence begun.
10	Many parting.
13 & 15	Separate individuals.
35	Immobile separate individuals.

Serum of horse immune against pig erysipelas.

Examined during 40 minutes; the trypanosomes did not agglutinate.

Serum of bull immune against septicaemia.

1	Trypanosomes slowly unite into small groups.
2	Small rosettes (3–5 individuals in each).
3	Rapid divergence.
6	Separate individuals.
8	Ditto.
12–40	Separation.
42	Final separation.

Serum of bull immune against chicken cholera.

1	Trypanosomes active.
2	Not agglomerated.
4	Agglomeration per 2–3 individuals; rosettes absent.
5	Divergence.
7–28	Separate individual exhibiting lively movements.
36	All separated.

VII. INFECTION OF ANIMALS[1].

The Turkestan camel trypanosome will infect many species of mammals and also some birds. The course of the disease is acute in mice, rats and dogs; sub-acute in guinea-pigs, rabbits and horses; and chronic in large cattle and sheep.

[1] By W. L. Yakimoff, W. T. Wassilevsky, and N. A. Zwietkoff.

(*a*) *Mice.* These animals proved very susceptible to infection. As in the case of *Trypanosoma evansi*, however, the course of the infection shows certain peculiarities. After being inoculated the trypanosomes appeared in the circulation of the mouse in a few days, but soon afterwards disappeared for a certain period. Later as a general rule they reappear, but in certain cases the blood remains free from parasites. Nevertheless such blood is infective as may be shown by sub-inoculations, and moreover the spleens of such animals are always hypertrophied. The following are records of the course of the infection in certain mice:

Mouse of 5th passage: infected on 29 April.

May 2 Trypanosomes appeared in the blood (+ v.f.)*.
„ 3–6 = +.
„ 6–9 = 0.
„ 10 = (+ v.f.).
„ 11 = +.
„ 12 = ++.
„ 13 = +++.
„ 14–15 = +.
„ 16 = +++.
„ 17 = ++++ (killed).

Mouse No. 2. Infected from guinea-pig on April 2nd.

April 3–15 = 0.
„ 10 = (+ v.f.).
„ 17 = 0.
„ 18 = (+ v.f.).
April 19–May 19 = 0. Observation interrupted.

Mouse No. 3. Infected from the same source as mouse No. 2 on April 2nd.

April 3–15 = 0.
„ 16 = + f.
April 17–May 20 = 0.
May 20 died. Spleen very hypertrophied.

Similar results are obtained by inoculating mice with infected blood from larger animals in which the infection is chronic.

Mouse No. 30. Inoculated subcutaneously from a calf (trypanosomes present in the blood of the calf), October 7th.

Oct. 8–21 = 0.
„ 22 = (+ v.f.).
„ 22–26 = 0.
„ 27, 28 = (+ v.f.).
„ 29 = 0.
„ 30 = (+ v.f.).
„ 31 = 0.
Nov. 1 = +.

* v.f. = very few; f. = few.

Mouse No. 87. Inoculated intraperitoneally with infected blood from a calf on Nov. 12 (trypanosomes absent).

Nov. 13–Dec. 2 = 0.
Dec. 3 = (+ v.f.)*.
„ 4–10 = 0.
„ 11, 12 = + f.
„ 13–15 = +.
„ 16 = ++.
„ 17–20 = +++.
„ 20–21 = ++++.
Dec. 22 died. Spleen greatly enlarged.

* v.f. = very few; f. = few.

Later both the period of incubation and the duration of the disease have shortened. On inoculating into the peritoneal cavity the period of incubation lasts as follows:

Bokhara strain	3–4 days
Termezh „	3–5
Samarkand „	5–9

By subcutaneous inoculation:

Bokhara strain	5 days
Termezh „	4

It is easily seen that in cases where the trypanosomes in the inoculated fluid are more numerous, the incubation period is shorter; if they are fewer, or invisible, this period is prolonged.

Death occurred 6–9 (in one instance 11, and in another 13) days after intraperitoneal inoculation, and 8 days after subcutaneous inoculation; and after the appearance of trypanosomes in the blood: 2–5 (and once 10) days after intraperitoneal, and 3–4 days after subcutaneous inoculation.

However we succeeded in invigorating our virus, so that the incubation period was reduced to 1 day, and death occurred 1–6 days after inoculation, and 3–5 days after the appearance of trypanosomes in the blood. At first, even after intraperitoneal inoculation, the period of incubation was 2 days, but later it did not exceed 1 day even after subcutaneous inoculation. Inoculation was always (except in mice Nos. 214, 220 and 227) performed with such a mixture of the trypanosomes in citrate, that each field of the microscope (Leitz, oc. 4, obj. 7) contained 1 trypanosome.

With the exception of the cases noted above, the parasites in mice multiplied incessantly to the maximum degree ++++.

No clinical phenomena were observable in mice infected with this trypanosome. The alterations in blood are dealt with on p. 69.

(*b*) *Rats.* When our virus had not yet become acclimatised in the laboratory animals, the inoculation of white rats produced the same results as in white mice. But later, when it was invigorated, the period of incubation

was reduced to 3 days (5–7 days in the absence of parasites in the blood) after intraperitoneal inoculation, and 4 days after subcutaneous inoculation; death occurred in 8–10 days after infection.

No symptoms of the disease were observed during life.

Grey rats are also easily infected. The incubation period, after inoculation with virus invigorated through passage in mice, is 4 days. The number of trypanosomes increased every day. Death took place on the 9th day after infection and 5 days after the appearance of the parasites in the blood.

(*c*) *Rabbits.* For these animals the trypanosomes appear in the blood after the following periods:

4–13 days after intravenous inoculation.
4–7 ,, ,, intraperitoneal ,,
10–13 ,, ,, subcutaneous ,,

Death occurred:
on the 46th day after intravenous inoculation.
,, 13th ,, ,, intraperitoneal ,,
,, 71–80 ,, ,, subcutaneous ,,

After the appearance of trypanosomes in the blood:
on the 35th day after intravenous inoculation.
,, 30–70 ,, ,, subcutaneous ,,

On two rabbits we observed the temperature curve:

Rabbit No. 1. Inoculated subcutaneously on March 12th, T. 39·0° C.

On the 13, 14, 15, 16, 17 and 18th the temperature rose progressively to 39·8°. On the 19th it was 39·1°, later again a rise to 39·8° (21st). On the 22nd till April 26th trypanosomes appeared in the blood, T. 39·6°. Later the temperature was between 39·8° and 40·5°. On the 27th and 28th April the temperature was 39·6° and on the 29th the rabbit succumbed.

Rabbit No. 2. Inoculated into the vein on the 12th March; T. 38·8° C. On the following days the temperature fluctuated between 38·7° and upward. On the 20th, when trypanosomes appeared in the blood, the temperature rose to 40·1°.

Until the first days of April the curve fluctuated in the limits between 39·5° and 40·6°; after that period it ranged between 38·8° and 40·6°.

The day before death it was 39·2°.

In *rabbit No.* 4 the temperature once reached 41·2°.

In nearly all our rabbits we succeeded in establishing the presence of trypanosomes in the blood. The quantity of them was never great—from + v.f. to + and only in one case ++++. The parasites rarely appear in the blood.

During the disease the rabbits emaciate considerably. No oedema is observed. The blood becomes pale.

(*d*) *Guinea-pigs.* It is in these animals that our virus from Turkestan camels was chiefly maintained.

The period of incubation depends on the quantity of trypanosomes introduced, and as in mice there is always a larger number of parasites than in other animals, the guinea-pigs inoculated from mice are infected earliest.

After inoculation of the blood from mice infected with the "Bokhara" virus—intraperitoneally—trypanosomes appear in the blood after 3–4 days; subcutaneously—after 4 days.

On the other hand, inoculating with the blood of mice infected with the virus of the "Samarkand" and "Termezh" strains, the period of incubation lasted from 4 to 6, and even 10 days. This is due, in our opinion, to the fact that the "Bokhara" virus had been invigorated by passage through mice for a long time, and had attained a high degree of virulency, whereas the virus of the "Samarkand" and "Termezh" strains had not been invigorated in this manner.

After infection from the blood of mice, the guinea-pigs succumb in 13–23 days, and after the appearance of trypanosomes in the blood—in 10–19 days (in one case the animal succumbed in 3 days).

On inoculating guinea-pigs with infected blood from other guinea-pigs, trypanosomes appear in the circulation in 5–12 days after intravenous inoculation (in one case the period of incubation lasted 23 days), and 12 days after subcutaneous inoculation.

Death occurred 10–33 days after inoculation, and 4–21 days after the appearance of trypanosomes in the blood.

Lastly, a certain proportion of the guinea-pigs were infected by means of blood from rabbits ("Samarkand" virus).

Here the trypanosomes appeared in the blood 4–17 days after intraperitoneal inoculation, and the animals succumbed 19–21 days after infection and 7–15 days after the appearance of the parasites in the blood.

Summarising the above, it may be assumed that the course of the infection varies according to (1) the method of infection, (2) the quantity of trypanosomes introduced and (3) the virulence of the virus. Trypanosomes appear in the blood of the guinea-pig in 3–17 days, and death occurs 13–33 days after infection and 4–21 days after the appearance of trypanosomes in the blood.

Trypanosomes are not always present in the blood of infected guinea-pigs. During the whole period of infection no external symptoms of the disease are observable.

(*e*) *Dogs.* These animals are very easily infected with trypanosomes.

In one dog (No. 1) we carried on detailed observations on the temperature:

At the very beginning (even at the first appearance of trypanosomes in the blood and during three days) it only rose a few tenths of a degree. On the 9th day (previously to the third appearance of the parasites in the blood) it rose above 40° C. Thence, from the 10th day, till the 18th it was below 40°, and once (on the 11th day) it fell even to 38·1°. On the 19th day it rose to 41°, to fall, however, on the next day to 38·8°.

Later the curve rose to a maximum of 41·5°, after which it remained

between 38·9°–40·8°. Finally the animal succumbed. After intraperitoneal inoculation the trypanosomes appear in the blood after 4 days, and after subcutaneous treatment, according to the amount of virus introduced, after 5–8 days.

The quantity of trypanosomes varied from + v.f. to +++.

The parasites are nearly always present in the blood.

In dog No. 1 the trypanosomes appeared on the 5th day but disappeared during the succeeding 2 days.

On the 8th day they were visible in the blood, disappearing on the 9th, but from the 10th to the 14th they were present.

On the 15th day they were absent, but were present from the 16th to the 22nd.

On the 23rd day they were absent.

From the 24th till the 26th they were present, but on the 26th and 27th they were absent.

Thence they were present from the 30th till the day of death (35th), increasing in numbers all the time.

As we have seen, from the time of the appearance of trypanosomes in the blood up to the day of death there were five brief periods (1–2 days) during which no trypanosomes were present in the blood. They were, however, indubitably present, but were so scarce, that it was impossible to detect them with the microscope.

Thus, the appearance of trypanosomes in the blood and their numbers correspond with the rises of temperature. There is no doubt that the temperature is due to the toxin produced by the trypanosomes.

In the days of high temperature the animals are dull, feed badly and are indifferent to their surroundings.

During the disease they emaciate considerably, so that sometimes they present the aspect of a skeleton covered with skin. Dog No. 1 was reduced in weight during 35 days of disease from 22 lbs. to 15½ lbs.

Oedemas are always present, chiefly on the abdomen and also on the head (oedema of the eyelids). Keratitis is often observed. Sometimes pareses and diarrhoea occur. Death occurs in complete apathy. The blood becomes pale (oligocythaemia, oligochromaemia). (For a special investigation of the blood of dog see below, p. 63.)

(*f*) *Sheep.* Sheep are also susceptible to infection with this camel trypanosome.

One ram was infected subcutaneously on the 12th March, 1917.

The trypanosomes first appeared in small numbers after 21 days, then disappeared and never re-appeared in the blood.

The temperature rose from the initial 39·5° to 40·3° in 6 days after infection, then fluctuated between 39·2° and 40·8°, but was more regular in the 4th month (fluctuations between 38·9°–40·3°).

We cannot state whether this ram had acquired immunity, as it succumbed in our absence.

(*g*) *Swine.* On the 20th October, 1914, a young pig was inoculated subcutaneously with the blood of an infected foal (quantity of trypanosomes + f.).

Trypanosomes appeared in the blood after 21 days and were again observed twice: on the 53–54th day, and on the 71–72nd day. During the rest of the time they were invisible.

The animal succumbed 73 days after infection.

The pig was emaciated.

(*h*) *Horses.* These animals were easily infected with this trypanosome.

We have inoculated one foal subcutaneously. The average temperature during the 8 days preceding the infection was 37·8° (from 36·9° to 38·4°).

After infection it rose on the 8th day to 40·5°, but next day it fell to 39·1°, and on the third to 38·4°.

Later it rose above 40° seven times:

On the 23rd day to 40·2°,
„ 31st „ 40·1°,
„ 46th „ 40·9°,
„ 60th „ 41·0°,
„ 68th „ 40·3°,
„ 76th „ 40·1°,
„ 81st „ (day before death) to 40·5°.

During the remaining time it was below 40°, but nearly always above the average; only twice did it fall below:

on the 58th day to 36·8°,
and „ 64th „ 36·4°.

On microscopical examination of the blood trypanosomes appeared for the first time on the 19th day after infection. However, a mouse inoculated on the 9th day (*i.e.* on the next day after the first rise of temperature) with a small amount of blood from the foal into the peritoneal cavity became infected (trypanosomes appeared in the blood on the 5th day, and the mouse succumbed on the 7th). Similarly, two guinea-pigs inoculated intraperitoneally on the 12th and 17th days of the disease became infected (trypanosomes appeared in the blood on the 8th and 10th days).

From these results we may conclude that trypanosomes were present in the blood long before they could be found on microscopical examination; probably, they were already present on the 8th day after the foal was infected.

During the whole course of the disease the parasites appeared in the blood during seven periods:

1st	period	during	4	days (18–22nd day)
2nd	„	„	5	„ (31–35th „)
3rd	„	„	6	„ (41–46th „)
4th	„	„	4	„ (57–60th „)
5th	„	„	2	„ (67–68th „)
6th	„	„	4	„ (73–76th „)
7th	„	„	1	„ (80th „)

The numbers varied from + v.f. to ++.

Their appearance in the blood was always accompanied by a rise in temperature.

During the disease the blood was virulent for laboratory animals. Thus, besides the above mentioned animals infected on the 9th, 12th and 17th days after inoculation of the virus into the foal, the following were infected:

(1) On the 33rd day: *mouse No.* 63 (subcutaneously, trypanosomes appeared in the blood after 3 days), and *rabbit No.* 4 (intraperitoneally; trypanosomes appeared in the blood after 4 days).

(2) On the 43rd day: *rabbit No.* 5 (intraperitoneally; trypanosomes appeared in the blood after 14 days).

(3) On the 57th day: *guinea-pig No.* 92 (trypanosomes appeared in the blood after 8 days).

(4) On the 70th day: *rat No.* 1 (trypanosomes appeared in the blood after 5 days).

The external phenomena of the disease in the foal were expressed in an icteric coloration of the conjunctiva and lip and spotty petechiae on the conjunctiva of brief duration, a suppurative discharge from the eyes and reddening of the eyelids.

During the second half of the disease (from the 47th day) the foal began to lie down sometimes; in the last third it remained lying down most of the time; and from the 66th day onwards it could hardly stand on its legs.

No oedemata were observed during the infection, but 6 days before death there was a swelling of the heel articulation of the right hind leg. Toward the end the appetite was greatly reduced and the animal was apathetic. It succumbed 82 days after infection.

The data concerning the investigation of the blood are given on p. 52.

(*i*) *Large cattle.* On the 23rd September, 1914, a young bull was inoculated subcutaneously with the blood of guinea-pig No. 81.

Trypanosomes appeared in the blood for the first time after 18 days, but mice inoculated with the bull's blood after 14 and 17 days respectively contracted the infection.

After the appearance of the parasites in the blood, they were visible for 4 days, but disappeared afterwards and were no longer seen.

However a mouse inoculated with the bull's blood 48 days after the in-

fection had appeared became infected. Later, guinea-pigs inoculated after 97 and 125 days did not become infected.

This bull, therefore, had suffered from trypanosomiasis but had recovered from the infection.

However, this did not render him immune.

For example, the serum taken 130 days after infection and mixed in different proportions with the same trypanosome from the "Bokhara" camels (as well as with the trypanosomes of Bokhara donkeys, which have already been proved to be identical with those of the camel) did not protect mice from infection after intraperitoneal inoculation, as seen in the following table:

Trypanosomes from camel	Quantity of serum	2nd day	3rd day	4th day	5th day
271	1·0 c.c.	0	0	+	
272	0·5	0	0	+	
273	0·25	0	0	+	
Trypanosomes from donkey					
268	1·0 c.c.	0	0	0	+
269	0·5	0	0	0	+
270	0·25	0	0	0	+

143 days after the first infection the bull was inoculated subcutaneously for the second time with the blood of a mouse with a larger number of trypanosomes (10–15 parasites per field) and in 4 days the trypanosomes made their appearance in the blood, but later they disappeared.

After the first introduction of the virus the temperature rose from 38° to 40° in 3 days, and later, during 20 days, it rose to between 38° and 41° and higher. Afterwards it became more regular and fluctuated between 39 and 40°. No symptoms of the disease were observed.

VIII. THE INFECTION OF BIRDS[1].

The following birds have been infected: goose, fowl, duck and pigeon.

(*a*) *Goose.* Inoculated intravenously on 16 October, 1914, from mouse No. 22 (quantity of trypanosomes ++++). Its blood was examined during 37 days; on the last day the bird succumbed, and during the whole period trypanosomes were never observed in the blood. Nevertheless, the goose proved to be infected, for two mice inoculated—one after 7 days (subcutaneously), and another after 21 days (intraperitoneally)—became infected.

(*b*) *Fowl.* On the 16 October, 1914, inoculated intravenously from the same mouse as the goose. Examination of the blood during 26 days did not reveal the presence of trypanosomes in the blood. Two mice inoculated with its blood—after 7 and 24 days respectively—did not become infected (examinations were conducted during 34 days).

[1] By W. L. Yakimoff and S. K. Popoff.

The fowl was again infected from the mouse inoculated with the blood of the goose on the 13 November, but did not contract infection: a mouse inoculated from it after 7 days did not become infected.

(*c*) *Duck.* On the 13 November a duck was inoculated intravenously with the blood of an infected mouse.

No trypanosomes were observed in the blood. Two mice inoculated intraperitoneally with its blood after 7 and 15 days did not contract infection (examination conducted during 21 and 18 days).

(*d*) *Pigeon.* On the 16 October, 1914, inoculated intravenously with the blood of an infected mouse.

During 26 days no trypanosomes were observed in the blood. Two mice inoculated with its blood—one after 6 days (subcutaneously) and the other after 16 days (intraperitoneally)—did not contract infection (examination conducted during 34 and 32 days).

26 days after the first infection the pigeon was reinoculated in the pectoral muscle, with a large quantity of trypanosomes. On the succeeding days there were no trypanosomes in the blood, and a mouse inoculated with the blood of the bird 7 days after the second and 33 days after the first, inoculation did not become infected.

Thus, of the four birds on which we experimented, only the goose proved to be susceptible to infection.

IX. ALTERATIONS IN THE BLOOD OF THE HORSE[1].

A foal was inoculated subcutaneously with the Bokhara strain of trypanosomiasis on 18. ix. 14. The animal remained infected for 81 days and during this period the following were examined:

(1) Red blood corpuscles.
(2) Haemoglobin.
(3) White blood corpuscles.
(4) Leucocytic formula.
(5) Arneth's Index.
(6) Alkalinity of the blood.

(1) *Red blood corpuscles.* Previous to infection the number of red corpuscles was 8,520,000 per cub. mm. Subsequent counts showed a marked diminution in the number of these elements during the course of the infection.

On the 13th day they numbered 7,136,000 per cub. mm.
„ 17th „ „ 7,120,000 „
„ 30th „ „ 4,960,000 „
„ 35th „ „ 4,256,000 „
„ 43rd „ „ 4,056,000 „

[1] By W. L. Yakimoff and W. T. Wasilevsky.

On the 51st day they numbered 3,680,000 per cub. mm.
„ 65th „ „ 3,456,000 „
„ 72nd „ „ 3,368,000 „
„ 79th „ „ 2,608,000 „

Thus the erythrocytes have decreased to 28·85 % of their original number. Besides the quantitative change, there is also a change in quality.

Beginning from the 10th day after infection, *agglutination* of the red blood corpuscles is observed, from the 13th—*normoblasts*, and from the 17th —*polychromatophilia*.

(2) *Haemoglobin*. The percentage of haemoglobin was estimated daily. Prior to infection it amounted to 80 % and already on the 5th day of the disease it had dropped to 70 %. Later the percentage diminished more gradually.

From the 5th till the 6th day after infection—70 %
„ 7th „ 8th „ „ —65
„ 10th „ 16th „ „ —60
„ 17th „ 22nd „ „ —55
„ 23rd „ 45th „ „ —50
„ 47th „ day of death —45

thus 43·7 % of the amount of haemoglobin was lost.

Colour index. Assuming the number of red blood corpuscles in this animal to be equal to 1, and the amount of haemoglobin also equal to 1, the colour index would be equal to 1.

After infection, during the first half of the disease, the index was less than 1:

On the 8th day—0·7
„ 13th „ —0·9
„ 18th „ —0·8

But from the 36th day it began to increase; on this day it was equal to 1·1.

After 36 days it was 1·1
„ 73 „ „ 1·5
„ 57 „ „ 1·5
„ 72 „ „ 1·5
„ 79 „ (3 days before death) 1·8

Such a difference is due to the fact that during the first half of the infection the diminution of the haemoglobin percentage proceeded more rapidly than the decrease in red blood corpuscles; whereas, during the second half, the relations were reversed: the red blood corpuscles were being destroyed more rapidly than the remaining elements dehaemoglobinized.

(3) *White blood corpuscles.* The most interesting phenomena observed relate to the leucocytes. On the day after infection their number rose from an average of 10,207 per cub. mm. to 17,105, and remained at this height three days longer. On the sixth day the curve descended to the normal and up to the 15th day it remained on this level, or even lower (to 5,725).

From the 15th day (4 days before the appearance of trypanosomes in the blood) the curve began to rise, until on the 17th day (one or two days before the appearance of trypanosomes) it reached the number of 17,853.

Later there came again a period of decrease, until the 27th day, when a new rise occurred, and a day before the second appearance of trypanosomes in the blood (which took place on the 30th day) the curve rose to 18,480.

In this second period of the appearance of trypanosomes in the blood the curve, which had fallen on the first day of this period to the normal, again began to ascend, and on the day following the disappearance of the trypanosomes it reached the highest point during the whole course of the disease (24,641).

After this the curve began to descend during the 4 succeeding days, to rise again on the first day of the third period of appearance of trypanosomes in the blood (41st day) to 20,720, and further—even to 22,659 (43rd day).

In the interval between the 47th and 57th days, when no trypanosomes were present in the blood, the number of leucocytes fluctuated between 12,269 and 17,563, but after the last date (49th day) tended to diminish.

With the next—fourth—appearance of trypanosomes in the blood (57th day) an increase in the number of leucocytes was again observed (57th day, 14,560, 58th, 15,222 and 59th, 15,529).

Further there were several more rises of the curve of leucocytes, sometimes accompanied by the presence of trypanosomes in the blood, and sometimes not.

From the above it may be assumed that during trypanosomic infection in the foal there occur four periods of leucocytosis:

(1) Initial, short, during 4 days (2–5 days), due to the spread of the infection in the organism.

(2) Also short, coinciding with the appearance of trypanosomes in the blood.

(3) The longest, during 39 days (from 28th till 66th day). This whole period may be called the period of leucocytosis, notwithstanding that the curve descended twice to the normal, and even lower.

(4) A shorter period of leucocytosis during 12 days (from the 70th till the day of death), in the course of which there were two falls, and the curve in general was not so high as in the third period.

The proportion of leucocytes to red blood corpuscles before infection was 1 : 840. Later, in the first period of the disease it increased (1 : 1240—on the 13th day), but as the infection advanced the ratio was less than the initial:

On the 17th day = 1 : 398
„ 30th „ = 1 : 268
„ 35th „ = 1 : 220
„ 43rd „ = 1 : 179
„ 57th „ = 1 : 286
„ 65th „ = 1 : 244
„ 72nd „ = 1 : 237
„ 79th „ = 1 : 112

This shortage is due to simultaneous oligocythaemia and leucocytosis.

(4) *The leucocytic formula.* Previously to infection the forms of leucocytes in this foal were distributed as follows:

lymphocytes	37·3 %
mononuclears	1·0
transitionals	4·2
polynuclears	45·7
eosinophils	6·5
mast cells	0·3
myelocytes	0·8
Türck's Reizungsformen ...	3·7

During trypanosomic infection the most conspicuous rôle belongs to the lymphocytes and neutrophil polynuclears.

Lymphocytes. On the day of infection, several hours after the inoculation of blood containing trypanosomes, the percentage of lymphocytes began to increase and from this day the curve began to rise with some fluctuations, reaching 70·3 % on the 19th day (the day of appearance of trypanosomes in the blood). Having fallen in the next 3 days to 43·3 %, 50·6 % and 36 %, on the 26th day the curve again rose to 62 %, and later, during the whole time up to the death of the animal, it stood high (to 74·6 % on the 36th day), only descending twice to 31 % and 21 %, respectively.

The curve of the *neutrophil polynuclears* presents the reverse of the curve of the lymphocytes: whilst the latter rises, the curve of the neutrophils falls, and *vice versa*. On the day of the highest lymphocytosis (74·6 %) there were only 7 % of polynuclears (on one occasion—on the 23rd day—the percentage was even lower—4 %).

During the whole period of the disease the percentage of the polynuclears was above the average; previously to infection only three times (on the 7th and 8th days—52·3 % and 47·3 %, on the 13th—52·6 %, and on the 73–75th —52 %–62 %), and on the two last days preceding death (46 % and 45·3 %).

The first rise (7th and 8th days) indubitably occurred on the day of penetration of the trypanosomes into the blood (mouse No. 21 inoculated with the blood of the foal on the 9th day became infected). This increase of the polynuclears may be termed the *period of primary polynucleosis.*

The second considerable rise of the curve of the polynuclears occurred on the 73–75th days (52–62 %), shortly before death. This period may be called the *period of secondary polynucleosis.*

During the remaining period the curve was below the average.

The *large mononuclears* do not appear to take any conspicuous part in the changes of the blood, but in general their percentage rises (to 6 %).

A more important rôle belongs to the *transitional forms.* From the mean of 4·2 % they rise in general, and sometimes even attain a high number (25·6 % on the 13th day), these rises of the curve corresponding to the presence of trypanosomes in the blood.

The comparatively high percentage of the *eosinophils* (6·5 %) before infection must have been due to the presence of a large number of ascarids in the intestine. Later this percentage rose only on the first two days preceding infection, whilst later it fell, even disappearing from the blood.

The *mast cells* which were present in insignificant numbers (0·6 %) before the infection, were always encountered in the blood beginning from the 15th day and their numbers even reached 6 %.

The percentage of *Türck's Reizungsformen*, prior to infection—3·6 %, not only did not rise, but even fell to 0·3 %.

The *myelocytes*, previously to infection 0·8 %, disappeared at the beginning of infection; but from the second third of the disease they reappeared (reaching 2·6 %) and remained till the 61st day, when they disappeared for 12 days; but from the 73rd day they reappeared and were present in the blood until the death of the animal. No correlation between the number of these elements and the presence of trypanosomes was observed.

In Yakimoff's work on the change of the blood in dogs infected by *Trypanosoma brucei* and *equinum* we see that during infection the curve of the total number of white blood corpuscles is directly related to the curve of the polynuclears, and inversely, to the curve of the lymphocytes, but there is not such a strict parallelism in this case.

Thus, the general picture of the distribution of the different forms of leucocytes during infection is as follows:

First period. Primary nucleosis with a diminution of the percentage of lymphocytes.

Second period, the longest, is characterized by mononucleosis (chiefly by lymphocytosis) with an increase in the mast cells and a decrease in polynuclears, both neutrophil and eosinophil.

Third period. Secondary polynucleosis (greater than the primary) with a corresponding diminution in the percentage of lymphocytes.

(5) *The Arneth Index.* Some interesting data were also obtained by the *Arneth* index.

The average number of the different forms of polynuclears prior to infection was as follows:

2-nuclears	...	...	21·1 %
3-	,,	...	38·7
4-	,,	...	29·9
5-	,,	...	7·1
6-	,,	...	3·8

Binuclears. During the first two or three days following infection there was a slight decrease in percentage, but beginning from the 5th day a progressive rise of the curve was observed, which rose higher and higher, reaching on the 21st day the maximum of 50·6 %.

With one or two exceptions a somewhat high proportion was maintained until the death of the animal.

Trinuclears. Already on the 3rd day after infection these tended to increase, and on the 43rd day reached the maximum (62·5 %). Several days later the curve began to fall, sometimes reaching the primary average, and even falling below; but 10 days before death it again rose (44–58·6 %) and remained so until death occurred.

In general, the curves of the bi- and trinuclears stand in inverse relations to each other.

Tetranuclears. These rose slightly on the second day after infection, but later during the whole period of disease they were below the normal, reaching 4·1 %, and further, although the curve rose sometimes, it never reached the normal. Only once (34th day) there was a sudden rise, when the curve reached 42·8 %, but the very next day it fell to 13·6 %.

Pentanuclears. Also increase slightly on the 3rd day after infection (to 12·1 %), but later during the whole period of disease they were below the normal, sometimes even altogether absent.

Hexanuclears. These follow the same order as the preceding polynuclears, whilst their disappearance from the blood is more definitely shown.

Summarising the changes in the *Arneth* index, we observe the following:

First period. On the first 2–3 days following infection there was a temporary decrease in binuclear polynuclears, the beginning of a rise of the trinuclears, and a slight rise of the tetra-, penta-, and hexanuclears.

Second period, the longest, terminating with the death of the animal, is characterized by the following: from the 5th day the percentage of the binuclears begins to rise; the trinuclears continue to rise; the tetra-, penta-, and hexanuclears remain below the normal and the 5- and 6-nuclears disappear from the blood at times.

Thus, the phenomenon took place known as the "shift to the left," termed "*anisocytosis*" by Arneth.

Therefore, comparing the alterations in the total number of leucocytes and in the Arneth index, the first period should be called *isohypercytosis*, and the second *anisohypercytosis*.

The destruction of leucocytes commences from the second day after in-

fection. From the primary average of 5·5 % the curve began to rise, and on the 21st day reached the maximum 36·3 %. Later the curve varied, but did not descend below 8·2 %, and only once rose to 25·3 %. The curve of leucocytosis was in inverse relation to the curve of the total number of leucocytes.

(6) *The alkalinity of blood.* Estimated by Engel's method, the alkalinity decreased visibly as the disease progressed, as seen in the following table:

Prior to infection	0·58455
On the 13th day after infection	0·56290
,, 25th ,, ,,	0·38970
,, 35th ,, ,,	0·34640
,, 51st ,, ,,	0·32475
,, 57th ,, ,,	0·34640
,, 66th ,, ,,	0·34640
,, 75th ,, ,,	0·25980

Thus, we observe in horses the same phenomenon, that had been witnessed by Yakimoff in dogs infected by trypanosomes, *i.e.* a decrease in the alkalinity of blood (53·8 %). However, Nierenstein, who revised Yakimoff's experiments, regards this phenomenon not as a decrease in alkalinity, but as an increase in the acidity of blood.

On considering this disease in the horse from the haematological point of view, we see that the blood picture is due chiefly to the following:

(1) olygocythaemia,
(2) oligochromaemia,
(3) leucocytosis,
(4) lymphocytosis,
(5) shift of the Arneth index to the left, and
(6) decrease in the alkalinity of blood.

The general picture of the life of the blood elements during trypanosomic infection in the horse may be represented as follows:

First period. From the day of infection till the appearance of trypanosomes in the blood.

Characteristic. Two primary leucocytoses. Beginning of leucocytolysis. Polynucleosis. Decrease in percentage of lymphocytes. Beginning of the "shift to the left" in the Arneth index. Beginning of decrease in the number of red blood corpuscles and the percentage of haemoglobin, and in the alkalinity. Decrease in the colour index. Widening of the correlation between the white and red blood corpuscles.

This period may be called the *period of the first lesser leucocytosis and polynucleosis.*

Second period. From the appearance of trypanosomes in the blood until the third period.

Characteristic. Leucocytosis of very long duration—the longest. Leucocytolysis. Lymphocytosis. Decrease in percentage of the polynuclears. Arneth index continues "shifting to the left." Continued fall in the number of red blood corpuscles, percentage of haemoglobin and alkalinity. Gradual rise of the colour index above 1. Narrowing of the correlation between the white and red blood corpuscles. Periodical appearance of trypanosomes in the blood.

This period may be called the *period of greatest leucocytosis and lymphocytosis.*

Third period. Several days preceding death.

Characteristic. Change in the increased and decreased number of leucocytes, terminating in the secondary—small—leucocytosis. Leucocytolysis. Second polynucleosis. Decrease in the percentage of lymphocytes. Arneth index continues "shifting to the left." Decrease to a minimum in the number of red blood corpuscles, the percentage of haemoglobin and the alkalinity. Continued rise of the colour index and a narrowing of the correlation between the white and red blood corpuscles. Periodical appearance of trypanosomes in the blood.

This period may be called the *period of the second leucocytosis and polynucleosis.*

Thus, the vital phenomena in the blood of the horse during experimental infection by trypanosomes are similar to those in dogs, with the difference, however, that in dogs the second period is characterized by leucopaenia, and in horses, on the contrary, by leucocytosis.

We shall try to consider these phenomena separately.

As was to be expected, the toxin produced by trypanosomes evokes an anaemic effect. The number of red blood corpuscles and amount of haemoglobin decrease.

The haematogenous organs are obliged to work intensely in order to supplement this deficit, and, therefore, we find in the peripheral blood normoblasts and polychromatophils (which some authors take for young red blood corpuscles). However, this struggle proves to be above their power—and toward the end of the life of the animal the number of red corpuscles and the percentage of haemoglobin fall.

The effect produced by this trypanosome is similar to that of the other trypanosomes studied in this respect (*Trypanosoma brucei, T. equinum, T. evansi, T. equiperdum*).

On taking up the work with the white blood corpuscles, we expected to meet with phenomena similar to those in dogs, in which (according to Yakimoff, Levi della Vida and C. Verdozzi), after a brief leucocytosis, during the whole period of infection and nearly till the very death, leucopaenia was observed. Instead of this, we see that the dominating phenomenon is leucocytosis. This phenomenon indubitably presents a reaction of the organism against infection.

Table 8.

Date	Erythrocytes	Leucocytes	Haemoglobin %	Lymphocytes %	Mononuclears %	Transitionals %	Polynuclears %	Eosinophils %	Mast cells %	Myelocytes %	Reizungsformen %	Alkalinity	Trypanosomes	Arneth index					Leucocytolysis %
														2 nuc. %	3 nuc. %	4 nuc. %	5 nuc. %	6 nuc. %	
15. ix.	8,520,000	10,131	—	41·6	1	4	40·7	7·3	—	1	4·3	0·58455	—	23·8	36·1	30·3	6·5	3	5·9
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
17	—	10,283	80	34	1	4·3	50·6	5·6	0·6	0·6	3	—	—	18·4	41·4	29·6	7·8	4·6	7·6
18	—	16,851	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	—	21,127	—	44·4	6·9	7·6	33·6	3·1	2·2	—	1·2	—	—	21·6	33	28·3	14·1	2·8	4·2
19	—	17,105	—	40·6	5	7	35·6	8·6	—	—	2·6	—	—	19·6	31·7	38·3	5·6	4·6	8·2
20	—	13,745	—	53	1·3	3·3	33	8	—	—	0·3	—	0	19·1	40·4	27·2	12·1	1	10·4
21	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22	—	16,596	70	52·3	0·6	3·3	36	5·6	—	0·6	0·3	—	0	29·6	42·5	23·1	5·5	—	16·8
23	—	10,385	70	48·6	1·6	3	39	5	—	—	1	—	0	24·7	46·1	23·1	4·2	1·7	11·7
24	—	6,516	65	41	0·6	2·6	52·3	2	—	—	1	—	0	28·6	46·4	19·7	3·8	1·2	19·1
25	9,016,000	8,501	65	42	5·3	3	47·3	0·6	—	—	1·6	—	0	28·8	45	16·2	7	2·8	19·1
26	—	10,742	65	54·7	2·3	7	35	0·3	—	—	0·6	—	0	27·6	52·9	13·3	4·8	0·9	18·2
27	—	9,062	60	44·3	3	16	30·6	2·6	0·3	—	3	—	0	35·8	43·4	14·1	6·5	—	27·5
28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29	—	7,789	60	47·3	3·3	5·3	40·6	0·6	—	—	2·3	—	0	27	49·1	20·4	3·2	—	20
30	7,136,000	5,752	60	35	3·6	4·3	52·6	1	0·3	0·3	2	0·56290	0	40·5	47·4	10·7	1·2	—	25·9
1. x.	—	9,723	60	49	1·3	8·3	39	0·6	—	—	1·6	—	0	38·4	47	11·9	2·5	—	17·1
2	—	12,065	60	53	1	13·6	27·6	3·6	0·3	—	0·6	—	0	43·3	42·1	13·2	1·1	—	17·8
3	—	15,374	60	60·3	7	11·3	19·3	0·3	0·6	—	1	—	0	31	50	15·6	3·4	—	15·8
4	7,120,000	17,853	55	65·6	1·3	8	20·3	1·6	1	—	2	—	0	45·9	31·1	21·3	1·6	—	13·5
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	—	9,011	55	70·3	1·6	4·3	22	—	1·3	—	—	—	+f.	34·8	43·9	18·1	3	—	19·7
7	—	12,014	50	43·3	2	16	30·3	3·6	1·3	0·6	2·6	—	+f.	36·2	43·9	18·6	1	—	25
8	—	7,280	50	50·6	3·7	15·6	27	0·6	1·6	—	0·3	—	+	50·6	44·4	4·9	—	—	36·3
9	—	10,945	50	36	1·3	13	42·6	2	4·6	—	0·6	—	+	35·9	46·8	12·5	4·6	—	23·6
10	—	9,927	50	62	6	25·6	4	—	0·3	—	2	—	0	25	58·3	16·6	—	—	16·6

11		—	7,687	50	65·6	3·6	16·6	8	3	2·3	—	0·6	—	0	45·8	50	4·1	—	—	22·2
12		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13		—	8,247	50	66·3	1·6	13·3	14	2·3	1·6	—	1·0	0·38970	0	40·4	33·3	21·4	2·3	2·3	19·1
14		—	11,505	50	60·3	1	8·6	22·6	2	3·3	0·3	1·6	—	0	45·5	45·5	5·8	2·9	1·4	24·8
15		—	14,152	50	58·3	2·6	7·6	19·3	3	5·3	1·6	1·3	—	0	36·2	37·8	22·4	3·4	—	15·9
16		—	13,185	50	60·6	3·6	11·6	18	0·3	3·6	0·3	1·3	—	0	44·4	44·4	11·6	—	—	21·2
17		4,960,000	18,480	50	57·6	0·6	7	24·6	1	2·6	2·6	1	—	0	32·4	48·6	14·8	4·1	—	22·8
18		—	10,029	50	59·6	2·3	6·6	23·6	1·3	2·6	0·3	1	—	+v.f.	28·1	54·9	15·4	1·4	—	10·9
19		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20		—	13,134	50	59·6	2	14·3	19	0·6	1·6	1	1	—	+v.f.	45·7	36·8	10·5	5·2	—	25·3
21		—	21,127	50	75	3·3	8·6	4·6	1·3	2·6	1	2	—	+	7·1	42·8	42·8	7·1	—	20·4
22		4,256,000	19,294	50	70·6	4	12·3	7·3	1·3	1	1·3	1	0·34640	+v.f.	27·2	54·4	13·6	4·8	—	17·8
23		—	24,641	50	74·6	2	8·6	7	3·3	1·3	—	1·3	—	0	38·1	38·1	23·8	—	—	23·4
24		—	16,902	50	67	3	6	16	3	0·6	1·3	0·3	—	0	29·1	47·9	22·9	—	—	15·2
25		—	19,753	50	69	0·6	3·6	18	1	3	1	1·6	—	0	42·5	42·5	12·9	1·8	—	19·7
26		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
27		—	13,342	50	68·6	2	6	17	1	1	1·6	2	—	0	21·5	52·9	21·5	3·9	—	15·2
28		—	20,720	50	65	2·6	7	14·6	2·3	2·6	2	1·3	—	+v.f.	22·7	50	25	2·2	—	14
29		—	18,369	50	57	1·3	9·3	19·6	1·6	6	—	1	—	+v.f.	35·5	44	20·3	—	—	12·7
30		4,056,000	22,659	50	72·3	2	6·6	10·6	1·3	2·3	1	1·3	0·30310	+v.f.	28·1	62·5	9·3	—	—	12·5
31		—	21,993	50	59·6	2·6	4·3	22·6	2	4	—	1·6	—	+	44·1	33·8	17·6	4·4	—	19·1
1.	xi.	—	8,603	50	72	1·6	8	10·3	1·6	2	0·6	2·3	—	+	32·2	58	9·6	—	—	9·1
2		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3		—	13,102	45	68·6	5·6	7·6	10·3	0·6	2	1·3	1	—	0	32·2	45·1	22·6	—	—	10·7
4		—	16,494	45	72·6	3·6	3·6	8·3	2·3	2·3	1	3	—	0	28	60	12	—	—	15·2
5		—	17,563	45	70·3	1·3	2	14·3	1	5	1	3·3	—	0	41·8	44·1	11·6	2·3	—	12·2
6		—	13,440	45	58·3	3·6	3·6	24·6	1·3	3	—	1·6	—	0	39·1	40·5	16·2	4	—	13·5
7		—	15,883	45	64	2·6	0·6	20·6	2·1	3·3	1·6	0·3	0·32475	0	37	41·9	20·9	—	—	12·2
8		—	13,287	45	67·6	1·3	3·6	20·6	1·6	1·3	0·3	1	—	0	41·9	40·3	17·7	—	—	17·1
9		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10		—	12,269	45	57·6	1·3	4·6	23·3	3	4·6	0·6	1	—	0	41·4	48·5	10	—	—	10·1
11		—	12,371	45	57·6	1	2·6	33	0·3	2·3	0·6	0·6	—	0	34·3	43·4	19·1	2·2	1	10·7
12		—	13,898	45	52·3	2	3	33·3	2	3	1·3	2·3	—	0	36	39	22	3	—	16·2
13		4,168,000	14,560	45	52·3	1·6	7·3	29·6	1·6	4	—	1·3	0·34640	+v.f.	35·9	40·4	17·9	4·4	1·1	8·8

Table 8 (*continued*).

Date	Erythrocytes	Leucocytes	Haemoglobin %	Lymphocytes %	Mononuclears %	Transitionals %	Polynuclears %	Eosinophils %	Mast cells %	Myelocytes %	Reizungsformen %	Alkalinity	Trypanosomes	Arneth index 2 nuc. %	3 nuc. %	4 nuc. %	5 nuc. %	6 nuc. %	Leucocytolysis %
14	4,184.000	15,222	45	58·6	3	2·3	23·6	1	4	1·3	2·6	—	+ +	28·1	53·5	14	4·2	—	8·2
15	—	15,529	45	59·6	6	10	10	—	1	—	2·3	—	+ +	36 6	38·3	18·3	5	1·6	12·2
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
17	—	10,640	45	71·6	2·6	6·6	16·3	0·6	—	—	1	—	0	28·5	48·9	18·3	4	—	13·5
18	—	18,633	45	69·3	0·6	2·6	21	2	2·3	—	0·6	—	0	46	28·5	23·8	1	—	16·8
19	—	12,625	45	68·3	1	6·3	16·6	2·3	1·3	—	2·6	—	0	28	42	24	2	4	19·7
20	—	16,291	45	56·6	0·6	5	31·3	2·3	0·3	—	2·3	—	0	34	37·2	22·3	4·2	2·1	14·2
21	3,456,000	14,152	45	51	1	7·3	33·3	2·6	1	—	1·3	—	0	38	46	14	2	—	16·6
22	—	17,716	45	31	2·3	8·6	47·6	3·6	2	—	3	0·34640	0	35·6	42·6	20·2	1·3	—	9·9
23	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24	—	10,385	45	65·3	2·3	4·3	24·6	0·3	—	—	2·1	—	+f.	47·2	36·4	14·8	1·3	—	7·4
25	—	10,080	45	58·6	5·3	21	11·3	1·3	—	—	2·6	—	0	35·2	44·1	17·6	2·7	—	19·3
26	—	14,458	45	64·6	1	12·3	16	4·3	0·6	—	1	—	0	43·7	35·4	16·6	4·6	—	16·7
27	—	14,152	45	50·6	2·6	5	33·3	2·3	3·3	—	2·3	—	0	27	44	24	5	—	15
28	3,368,000	14,203	45	51·3	2	5·3	35·6	3·3	0·6	—	1·3	—	0	25·2	55·1	15·8	3·7	—	12·7
29	—	7,025	45	36·6	1	5	52	2·2	0·3	0·3	1	—	+v.f.	17·9	55·1	21·1	5·8	—	20·4
30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1. xii.	—	14.967	45	21·3	2·3	7	62	—	3	2	1·6	0·25980	+ +	21·5	47·3	24·7	5·9	0·5	8·2
2	—	12,931	45	54	5	13·3	19·3	—	2	2·3	3	—	+	29·3	58·6	10·3	1·7	—	12·5
3	—	6,211	45	46·3	2	9	35·6	—	3	1	2·3	—	0	23·3	56	18·6	0·9	0·9	19·5
4	—	12,727	45	57	0·6	8	29·6	1	1	1	1·3	—	0	33·7	49·4	15·7	1·1	—	12·2
5	2,608,000	14,356	45	43·6	—	7·3	46	0·3	1·6	0·6	0·3	—	0	31·1	52·8	13·7	2·1	—	16·6
6	—	13,898	45	47·3	2·3	1·3	45·3	0·3	2	—	0·3	—	+f.	41·9	48·5	8·8	0·8	—	11·5
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

But here the question may arise: why do we observe in dogs leucopaenia, instead of leucocytosis? This can be explained as follows:

In the horse the infection bears a sub-chronic character; trypanosomes are not always present in the blood, and their number varies between + v.f. and only ++.

In the dog, however, this disease is acute; trypanosomes are always present in the blood and in large numbers.

It is on account of this difference that the organism of the horse struggles more successfully against the infection; the leucocytes fighting against the parasites pass into the blood in larger numbers than in the dog; therefore, they are all the time present in increased numbers, as if "on guard." In dogs, however, the parasites—the foes of the organism—are more powerful than its defenders, and the latter are fewer.

Nevertheless, many leucocytes also perish in the organism of the horse (which is seen in the enormous leucocytolysis observed by us) and the organism, through its haematogenous organs, is obliged to fill up the deficiency in the form of young elements—lymphocytes.

Now we can understand the reason of such an enormous loss of polynuclears, hence the "shift to the left" in the Arneth index, and the appearance in the blood of the "young" leucocytes—the lymphocytes—and their rapid transition into the "mature" (transitional) forms.

X. CHANGES IN THE BLOOD OF THE DOG DURING INFECTION WITH THE CAMEL TRYPANOSOME[1].

A dog was inoculated intraperitoneally with a large quantity of blood containing trypanosomes and as a result became infected after an incubation period of only 4 days. It succumbed on the 35th day. Trypanosomes appeared in the blood on the 5th day after infection (+) and after this for three periods:

from the 8th to the 14th day
,, 16th ,, 25th ,,
,, 29th ,, 35th ,,

the number of trypanosomes varied from + v.f. to +++.

The red blood corpuscles which previous to infection numbered 6,048,000 decreased progressively:

On the	5th	day	after	infection	there were 6,584,000
,,	11th	,,	,,	,,	5,544,000
,,	17th	,,	,,	,,	3,876,000
,,	21st	,,	,,	,,	3,496,000
,,	25th	,,	,,	,,	3,744,000
,,	35th	,,	,,	,,	2,728,000

By W. L. Yakimoff and R. N. Kohan-Marnier.

Besides quantitative changes, there are also observed qualitative changes such as polychromatophilia and appearance of normoblasts.

The total number of white blood corpuscles prior to infection was on the average 12,548 per cub. mm. On the day after infection the curve rose to 19,753, and on the 3rd day to 21,025. On the 4th day it descended to 11,506, but from the day following it again began to rise and on the 7th day reached the maximum number of 28,255. It remained at a high level till the 12th day, when it began to fall, reaching 8,754 (on the 14th day). Further rises and falls succeeded one another, in general showing a tendency to decrease, but several days preceding death a tendency to rise is observed, which reached 20,099 on the last day.

Thus, if we consider the curve of the white blood corpuscles, we may speak of three periods in the life of these elements.

A *first period* of primary leucocytosis occurring during the first days after infection, and during the first days of the appearance of trypanosomes in the blood.

A *second period* (the longest) is characterized by the number of leucocytes being in general close to the normal with a tendency toward leucopenia.

A *third period* (several days preceding death) should be called the period of secondary leucocytosis.

The *correlation between the white and red blood corpuscles* narrows:

Before infection	1 : 460
On the 5th day after infection	1 : 397
,, 11th ,, ,,	1 : 241
,, 17th ,, ,,	1 : 302
,, 21st ,, ,,	1 : 328
,, 25th ,, ,,	1 : 487
,, 35th ,, ,,	1 : 135

Forms of leucocytes. Before infection the different forms of leucocytes were distributed on the average as follows:

Lymphocytes	24·8 %
Mononuclears	2·6
Transitionals	4·3
Polynuclears	64·8
Eosinophils	0
Mast cells	0
Türck's Reizungsformen ...	1·2
Myelocytes	0·8

The chief rôle in the change of the leucocytic formula belongs to the lymphocytes and neutrophil polynuclears.

Immediately after infection the percentage of lymphocytes began to fall and on the 3rd day reached 1·3 %, but on the next day the curve began to

rise and on the 7th day reached its maximum, 36·6 %, falling on the following day to 13·6 %. Further, up to the 28th day the percentage of these elements varied between 15·0 % and 30·6 %, in general showing no tendency to fall. From the 29th day a distinct tendency to decrease was observed (amplitude between 8·3 % and 17·3 %).

Thus, in the life of these elements three periods are observed:

First period, decrease in percentage.

Second period, slight increase, and absence of a tendency toward the decrease in percentage.

Third period, second decrease in percentage.

The *neutrophil polynuclears* behave in the opposite manner, being in inverse relation to the lymphocytes. Whilst in the first period the curve of the lymphocytes falls, that of the polynuclears rises (to 95·6 % on the 3rd day); during the second period, whilst the number of lymphocytes increases slightly, the percentage of polynuclears falls (fluctuations between 56·6 % and 80 %), and although the curve sometimes rises above the average, no tendency toward polynucleosis is observed; lastly, during the third period (from the 29th day till death) the curve rises abruptly (to 91·6 % on the 33rd day), and on the day of death the percentage is 87.

Is the same phenomenon, which Yakimoff noticed in dogs infected with *Trypanosoma brucei* and *T. equinum*, observed here: the direct relation of the curve of the total number of white blood corpuscles to the curve of polynuclears, and inverse relation to the curve of the lymphocytes?

During the first 6 days such a phenomenon was observed, but later (from the 7th day till the 19th) the opposite was observed; the curve of the total number of white blood corpuscles was directly related to the curve of lymphocytes, and inversely to the curve of polynuclears; whilst from the 20th day onwards the former conditions were re-established.

As regards the large mononuclears and the transitional forms, during infection a decrease in their percentage is observed, and the former sometimes even disappear altogether from the blood.

No eosinophils and mast cells were present previous to infection, but they appeared later, the latter elements in the last two days of the life of the animal.

The percentage of *Türck's Reizungsformen* and myelocytes increases during the infection.

The Arneth index. The Arneth index had the following aspect before infection:

1-nuclears	...	...	...	81·1 %
2- ,,	...	...	...	17·0
3- ,,	...	...	...	1·3
4- ,,	...	...	...	0·5

The *mononuclears* rose to 90·3 % on the next day after infection. Further, up to the death of the animal the curve remained above the initial figure, the lowest percentage being 87·5, and the highest 97·5 (on the last day).

Table 9.

Days of Infection	Month and Date	°C.	Total number of erythrocytes	Total number of leucocytes	Correlation between leucocytes and erythrocytes	Lymphocytes %	Mononuclears %	Transitionals %	Polynuclears %	Eosinophils %	Mast cells %	Reizungsformen %	Myelocytes %	Arneth Index 1 nuclear %	Arneth Index 2 nuclear %	Arneth Index 3 nuclear %	Arneth Index 4 nuclear %	Trypanosomes	Weight lbs	Notes
—	14. v.	—	—	13,440	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
—	15	—	—	12,981	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
—	16	—	6,048,000	13,133	1 : 460	26	2	5·6	62·6	—	—	0·3	0·6	81·1	17·9	1·3	0·5	—	—	
1	17	39·4	—	10,639	—	23·6	3·3	3	67	—	—	2	1	—	—	—	—	—	—	Inoculated intraperitoneally with the trypanosomes of camel ("Bokhara")
2	18	39·3	—	19,753	—	3·3	—	3	89·3	0·3	—	—	4	90·3	8·5	1·1	—	—	—	
3	19	39·7	—	21,025	—	1·3	1	1·3	95·6	0·3	—	—	0·3	90·6	7·6	1·3	0·34	—	—	
4	20	39·2	—	11,506	—	12·3	2·6	2	81	0·3	—	0·6	1	88	9·8	1·6	0·4	—	—	
5	21	39·3	6,584,000	16,545	1 : 397	10·3	2·6	2·3	83·6	0·3	—	0·3	0·3	88·7	9·5	1·5	0·3	+	22	
6	22	39·7	—	13.949	—	17·6	1·6	3·3	74	0·3	—	2·3	0·6	88·7	9·5	1·3	0·4	0	—	
7	23	38	—	28,255	—	36·6	1·6	1·6	58	—	—	1·6	0·3	92·5	6·8	—	0·5	0	—	
8	24	38·9	—	15,069	—	13·6	6·3	2·3	75·6	—	—	1·6	—	92·1	6·2	1·3	0·4	+ +	—	
9	25	40·3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+ +	—	
10	26	39·7	—	14,865	—	17·6	0·3	3·3	78·6	0·3	—	—	3	91·9	6·7	0·8	0·4	+f	—	
11	27	38·1	5,544,000	22,960	1 : 241	25·6	2·6	3	62·3	0·6	—	2·6	3·3	94·1	3·1	1·06	0·5	f.+	22	
12	28	39·8	—	10,233	—	16·6	0·6	1·6	77·3	1	—	0·3	2·3	87·5	10·3	1·2	0·8	+ +	—	
13	29	39·4	—	12,218	—	15·7	2	2	75·6	2·3	—	1	1·3	94·2	4·4	—	1·3	+ +	—	
14	30	39·7	—	8,754	—	13·6	4·3	1·3	73·3	0·6	—	0·6	6	91·8	6·8	1·3	—	+	21½	
15	31	39·5	—	18,581	—	34·3	1·3	3	56·6	0·3	—	0·6	2·6	90	8·2	1·7	—	0	—	

16	1. vi.	38·6	—	19,217	—	—	—	—	—	—	—	—	—	—	—	—	—	+f.	—
17	2	39·1	3,876,000	12,829	1 : 302	15	1·6	1·3	80	—	—	0·3	1·6	92	9·5	1·7	0·4	+	—
18	3	40	—	10,996	—	22·3	0·3	2	67·3	—	—	4·6	3·3	92·5	6·4	0·9	—	+ +	21
19	4	40·9	—	15,527	—	17	0·6	4·3	75	—	—	3	—	89·2	9·2	1·2	—	+v.f.	—
20	5	38·9	—	9,673	—	30·6	—	1	58	—	—	3·1	3·6	95·4	4·1	—	—	+v.f.	—
21	6	39·4	3,496,000	10,640	1 : 328	21·6	0·3	1·6	72·3	0·6	—	1·6	1·6	93·5	6·4	—	—	+f.	—
22	7	40·3	—	12,931	—	16·3	—	2	76	0·3	—	2·6	2·6	91·2	7·4	0·8	—	+ +	—
23	8	40·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+ +	—
24	9	39·6	—	8,807	—	27	0·6	4	63	0·6	—	1·3	3·3	8·9	8·9	1·5	—	+ + +	—
25	10	40·2	3,744,000	7,687	1 : 487	19	0·3	1·3	72·6	0·6	—	1	2·6	96·7	2·7	0·4	—	+ +	19
26	11	41·5	—	6,676	—	25·8	1	2·6	67·6	—	—	1	2·6	96·5	3·4	—	—	+v.f.	—
27	12	39·3	—	14,000	—	28	—	0·6	71	—	—	1	—	91	6·5	0·9	—	0	—
28	13	38·8	—	8,909	—	25	—	1·6	71·6	—	—	0·6	1	93·4	6	0·4	—	0	—
29	14	39·1	—	18,351	—	16	—	1·3	81·6	—	—	0·3	0·6	96·3	2·8	0·4	0·4	+f.	—
30	15	39·5	—	15,120	—	14·3	0·3	1·3	83	—	—	0·3	0·6	93·9	4·8	0·8	—	+ +	17
31	16	39·2	—	11,403	—	15·3	—	0·6	83	—	—	0·3	0·3	95·6	4·4	0·4	—	+ +	—
32	17	39·8	—	7,585	—	17·3	—	0·6	82·3	—	—	0·3	—	96·3	2·8	0·4	0·4	+ +	—
33	18	39·7	—	16,902	—	8·3	—	—	91·6	—	—	—	—	95·9	3·2	0·8	—	+ + +	—
34	19	39·5	—	12,931	—	10·3	—	2·3	85	—	0·6	0·3	1·3	94·9	3·9	1·1	—	+ +	15¼
35	20	39·8	2,728,000	20,099	1 : 135	14	—	0·6	87	—	0·6	0·6	0·3	97·5	2·3	—	—	+ + +	—

The *binuclears* fell immediately after infection to 8·5 % and later never rose above 10·3 %; the lowest percentage was on the last day—2·3 %. The curve of these elements exhibited a fall.

The *trinuclears* all the time kept falling, and later disappeared altogether from the blood.

This fall and disappearance was still more definitely expressed in the *tetranuclears*, which were absent from the blood during 18 of the 36 days of infection.

Thus, in this dog, the "shift to the left" of the Arneth index was distinctly expressed.

Summarising these data, we may state that in the dog infected with the trypanosome of the Bokhara camels, the changes in the formed elements of the blood observed by us are as follows:

(1) Oligocythaemia.

(2) (*a*) Primary leucocytosis, (*b*) relative leucopenia, and (*c*) secondary leucocytosis, and

(3) "Shift to the left" of the Arneth index.

The general picture of the life of the blood elements may be divided into three periods:

First period. From the beginning of infection till the first appearance of trypanosomes in blood.

Characteristic. Primary leucocytosis. Polynucleosis. Decrease in the percentage of lymphocytes. Beginning of the "shift to the left" of the Arneth index. Beginning of the decrease in the number of red blood corpuscles.

Second period. From the second period to the third.

Characteristic. A tendency towards a decrease in the total number of white blood corpuscles. A tendency towards an increase in the percentage of lymphocytes and towards a decrease in neutrophils. The Arneth index "shifts to the left." The number of red blood corpuscles falls.

Third period. Several days before and up to death.

Characteristic. Secondary leucocytosis. Polynucleosis. Decrease in the percentage of lymphocytes and increase in neutrophils. "Shift to the left" of the Arneth index. Large fall of the red blood corpuscles.

Thus, we see that in dogs infected with the trypanosome of Turkestan camels the life of the formed elements of the blood shows the same phenomena, which were observed by the authors in dogs infected with *Trypanosoma brucei*, *T. equinum* and *T. gambiense*.

It is true, these phenomena are not so strongly expressed, as was the case in the observations of some authors (Yakimoff, Levi della Vida and Verdozzi), but we are inclined to explain this either by the difference of the trypanosomes, or by the method of infection. In general, however, the picture remains the same.

XI. CHANGES IN THE WHITE AND RED BLOOD CORPUSCLES IN MICE[1].

Mice were inoculated respectively with trypanosomes from camels passed through a guinea-pig, and trypanosomes from donkeys passed through a rat.

Table 10.

Mouse No. 16. Infected with camel trypanosomes.

Days	Number of erythrocytes per 1 cub. mm.	Trypano-somes	Notes
1	11,450,000	—	Infected from guinea-pig No. 81
2	11,456,000	0	
3	11,296,000	0	
4	9,056,000	0	
5	11,024,000	+	
6 and 7	—	+ +	
8	8,992,000	+ + +	
9	7,829,000	+ + + +	
10	—	—	Succumbed

Mouse No. 14. Infected with trypanosomes of donkey.

Days	Number of erythrocytes per 1 cub. mm.	Trypano-somes	Notes
—	8,202,000	—	
—	8,880,000	—	
1	8,640,000	—	Infected from rat No. 2
2	8,930,000	0	
3	7,808,000	+	
4	7,640,000	+ +	
5	5,592,000	+ + + +	
6	—	—	Succumbed

Table 11.

Mouse No. 11. Infected with trypanosomes from camel.

Days	Number of leucocytes per 1 cub. mm.	Trypano-somes	Notes
—	18,683	—	
1	21,178	—	Infected from guinea-pig No. 70
2	17,054	0	
3	14,967	0	
4	15,833	0	
5	43,222	0	
6	18,073	0	
7	18,785	+	
8	10,691	+ +	
9	10,691	+ + +	
10 and 11	—	+ + +	
12	10,487	+ + +	
13	10,691	+ + +	
14	14,524	+ + + +	
15	—	—	Succumbed

[1] By W. L. Yakimoff and W. N. Hamburger.

Table 11 (*continued*).

Mouse No. 13. Infected with trypanosomes from donkey.

Days	Number of leucocytes per 1 cub. mm.	Trypano-somes	Notes
—	14,916	—	
—	14,662	—	
1	—	—	Infected from rat No. 2
2	16,953	0	
3	18,785	+	
4	12,574	+ +	
5	8,247	+ + +	
6 and 7	—	+ + +	
8	16,851	+ + +	
9	10,600	+ + +	
10	14,254	+ + + +	
11	—	—	Succumbed

In the first case the trypanosomes appeared in the blood on the 5–6th day after infection, and in the second case on the third day. The two mice infected with the trypanosome from camels lived respectively 9 and 14 days after inoculation, and the two infected with the trypanosome from donkeys, 4 and 10 days after inoculation.

The number of trypanosomes in the circulation increased progressively each day and on the day before death reached the maximum of (++++).

The *red blood corpuscles* decreased in number after infection, and on the last day of life were respectively 31·8 and 31·6 % of the total number. Also in mice, more than in any other animals (except rats), are expressed the qualitative changes of the red blood corpuscles: *normoblasts*, *polychromatophilia* and *poikylocytosis*.

The changes in the total number of *leucocytes* were especially sharp in the mouse infected with the trypanosomes from the camel.

Before infection the average of these elements was:

(1) in animal infected with trypanosomes from camel 19,930,
(2) ,, ,, ,, ,, donkey 14,789.

In the first mouse the curve of leucocytes descended slightly after infection, but on the day preceding the appearance of trypanosomes in the blood it rose to the high figure of 43,222. On the next day the curve fell below the mean and later the number of these elements remained equal to nearly half the average number (slightly above 10,000). On the day before death the curve showed a tendency to rise (to 14,524).

A similar picture, but not so sharply expressed, was present in a mouse infected by the trypanosomes from donkeys.

On the day after infection and on the day preceding the appearance of trypanosomes in the blood, the curve rose from 14,789 to 16,953 and on the day of the appearance of the parasites in blood it showed 18,785. Later the

curve was below the mean, only on the 8th day of infection rising to 16,851. On the day before death there was again a rise of the curve (to 14,254).

Thus, we see that in mice the same phenomena are also observed as in dogs infected with trypanosomes, with the same three periods.

First period. From the moment of infection till the appearance of trypanosomes in the blood, characterized by primary leucocytosis.

During the *second period* leucopenia is observed.

During the *third period*, preceding death, there is a secondary leucocytosis, but smaller than the primary.

XII. PATHOLOGICAL ANATOMY[1].

The pathological changes in the animals that have succumbed as the result of experimental infection with the trypanosomes from camels are few.

In *mice* the hypertrophy of the spleen is very striking.

According to the data obtained by dissection of 96 mice, the spleen was greatly, sometimes enormously, enlarged in 66 (68·7 %); enlarged, not strongly, in 27 (28·1 %); and slightly enlarged in 3 (3·1 %). Regarding the changes in other organs a slight enlargement and hyperaemia of the liver was sometimes observed.

In *rats* also the spleen is greatly enlarged.

In *guinea-pigs* no marked hypertrophy of the spleen is observed. From the whole number examined in this respect the spleen was hypertrophied in 25 %; slightly enlarged in 50 %; and not enlarged in 25 %.

In *rabbits* the spleen is but slightly enlarged.

The data concerning the dissections of the *foal* are as follows[2]:

Slight emaciation. Under the skin on the neck and at the beginning of the breast, slight oedema of the connective tissue. On the right hind leg anteriorly to the heel articulation, a gelatinous oedema. In the *peritoneal cavity* about ½ litre of exudation of pink colour. In the *stomach*, numerous bot-fly larvae. In the *duodenum*, yellowish mucus, a great number of ascarids. Peyer's patches not enlarged, but reticulate. The *ileum*, empty. The *jejunum* contains a greenish pulp; on the mucous membrane there is yellowish mucus. In the *caecum*, on the sub-mucous membrane, there are nodes of parasitic origin (*Strongylus armatus*). Helminthic aneurism of art. coecalis. In the *intermaxillary space* and around the throat yellowish oedema. In the anterior part of the *thoracic cavity* there is a considerable infiltration of ochraceous colour under the pleura. In the thoracic cavity itself there is about ½ litre of fluid. The whole *pericardial membrane* is impregnated with a yellow, but denser, infiltration. On the transverse sulcus of the *heart muscle* there is a similar infiltration. In the right ventricle there are fresh clots of fibrin. The cardial valves are normal. The *lungs* are normal. The *liver* has a normal aspect; in section it is of a dark-brown colour. The *kidneys* are normal; the capsule is detached with difficulty. The *spleen* is normal. The *oesophagus*, *trachea* and *pharynx* are normal.

Thus, the phenomena of trypanosomic character occurring in the foal were as follows:

[1] By W. L. Yakimoff.

[2] We are indebted to M. T. Romanovitsch for the dissection of the foal and pig.

Oedemata of the subcutaneous connective tissue of the neck, beginning of breast and one articulation; *infiltration* of yellow colour under the pleura of the anterior part of the thoracic cavity, on the pericardium, transverse sulcus of the heart muscle and the intermaxillary space; *exudation* in thoracic and peritoneal cavities; and *emaciation*.

The data obtained by dissection of the young *pig* are as follows:

Emaciation in the *subcutaneous connective tissue*, no oedemata are present; the *lymphatic glands* of the lower jaw are somewhat enlarged, compact, with a reddish tint. In the *thoracic cavity* there is a serous exudation amounting to one table spoonful; in the *lungs* there are several compact foci about the size of a pea of gray-red colour; in the *pericardium* there is about one table-spoonful of yellowish exudation; in the *peritoneal cavity* there are about 1½ table-spoonfuls of light yellow exudation. The *spleen* is enlarged (17–18 cm. diameter), compact, under the capsule are visible protuberances slightly yellower than the remaining tissue, hardly protruding above the surface. In the *remaining organs* no anomalies are visible.

Thus the pig exhibited the following phenomena characteristic of trypanosomiasis: *emaciation*; *lymphatic glands* somewhat enlarged; in the *thoracic* and *peritoneal cavities*, and in the *pericardium*, the presence of exudation; the *spleen* enlarged, compact.

The dead *goose* exhibited no changes.

XIII. CHEMO-THERAPY[1].

In the experiments on the treatment of experimental trypanosomiasis in camels, we have tested the following drugs:

1. From the *arsenic group*: atoxyl, arsenosolvin, salvarsan, tartarus stibiatus (potassium stibiate) and trixidin.
2. From the *benzidine group*: trypan-blue and dianil-blue (or trypanil).

The experiments were carried out by us so as to determine first the dosis toxica, and then the dosis tolerata of the drug employed.

Then on the first day of the appearance of trypanosomes in the blood, the infected mice were injected with a solution of the drug, the strength of the solution being estimated so that each mouse received 1 c.c. per 20 gm. live weight. Injection was carried out intravenously with all the preparations, except trixidin.

Inoculations were made with the virus of Bokhara camels invigorated by passage through mice, the incubation period lasting at the most two days, and death taking place 3 days after the appearance of trypanosomes in the blood.

Experiments with atoxyl. The atoxyl employed was made by the Aktien Gesellschaft, Charlottenburg.

The results of the tests on the toxic qualities of the preparation are seen in the following table.

[1] By W. L. Yakimoff and W. T. Wasilewsky.

Table 12.

Solution	Results
1 : 150	Succumbed
1 : 200	Survived
1 : 300	
1 : 400	
1 : 500	
1 : 600	
1 : 700	
1 : 800	

Thus, for atoxyl the following results were obtained:

dosis laetalis: solution— 1 : 150.
dosis tolerata: solution—1 : 200.

The results of the treatment of mice infected with trypanosomes are given in Table 13.

Table 13.

	Days											
Solution	1	2	3	4	5	6	7	8	9	10	11	12
1 : 200	+v.f.⊕	0	0	0	0	0	0	0	0	0	0	recurrence
1 : 250	+v.f.⊕	+f.	0	0	0	0	0	0	recurrence			
1 : 300	+f.⊕	+	0	0	0	0	0	recurrence				
1 : 400	+⊕	++	++	+++	+++	death						
1 : 500	+f.⊕	+	++	+++	++++	,,						
1 : 600	+v.f.⊕	+f.	+	++	++	,,						
1 : 900	+f.⊕	+	++	++	death							
1 : 1200	+⊕	++	+++	death								

+v.f. denotes trypanosomes very few.
+f. = trypanosomes few.
⊕ = injection of the drug.

From this table it is seen that in doses approaching the toxic doses (1 : 200, 1 : 250, 1 : 300) atoxyl drives the trypanosomes out of the blood. When the solution is administered in the proportion 1 : 200 the trypanosomes disappear in blood on the next day; when 1 : 250 or 1 : 300 they disappear on the 3rd day after injection.

But this disappearance of the parasites from the blood is only temporary, as the parasites reappear after some time and the weaker the dose the quicker the reappearance, *e.g.* 1 : 200—after 11 days; 1 : 250—after 8, and 1 : 300 after 7 days.

As regards weaker doses, the trypanosomes are not driven out of the blood, but death is postponed for 2 or 3 days, and in still weaker doses the period is shortened.

If the trypanosomes are very numerous, even a solution of 1 : 300 does not expel the trypanosomes from the blood (see Table 14).

Table 14.

Solution	Days 1	2	3	4	5
1 : 300	+ v.f.	+ +	+ + +	+ + + ⊕	death
1 : 400	+	+ +	+ + + ⊕	+ + + +	„
1 : 500	+	+ +	+ + + ⊕	death	
1 : 600	+	+ +	+ + + +	+ + + ⊕	death
1 : 800	+	+ +	+ + + +	+ + + ⊕	„
1 : 1000	+	+ +	+ + + +	+ + + ⊕	„
1 : 1200	+	+ +	+ + ⊕	+ + + +	„

In this case death takes place on the next day, and rarely on the third day after injection. As is seen, a single injection of atoxyl, even in doses approaching the toxic, does not produce recovery.

Thus, this preparation is not suitable for the treatment of infection with the camel trypanosome.

Experiments with Tartar emetic. The toxicity of the potassium antimony tartrate at our disposal is shown in Table 15. The trypanocidal effect of the preparation is shown in Table 16.

Table 15.

Solution	Results
1 : 200 1 : 800 1 : 900	Succumbed
1 : 1000 1 : 1200	Recovered

Table 16.

Solution	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 : 1200	+ f. ⊕	0	0	0	0	0	0	0	0	0	0	0	0	0
1 : 1500	+ v.f. ⊕	0	0	0	0	0	0	0	0	0	0	0	recurrence	
1 : 1800	+ v.f. ⊕	0	0	0	0	0	0	0	recurrence					
1 : 2000	+ v.f. ⊕	0	0	0	0	0	0	recurrence						
1 : 5000	+ v.f. ⊕	0	0	0	0	0	recurrence							
1 : 8000	+ + ⊕	+ +	+ +	death										

Thus, an intravenous injection of 1 c.c. of a solution of 1 : 1200 of tartar emetic cures mice infected with trypanosomiasis.

Beginning from 1 : 1500 to 1 : 5000 recurrences occur, and the smaller the dose, the sooner the latter are produced.

A solution of 1 : 8000 does not drive the trypanosomes out of the blood even temporarily.

Since the toxic dose is 0·0011 gramme (in solution 1 : 900) per 20 gr. live

weight of the mouse, and the therapeutic dose is 0·0008 (in solution 1 : 2000), the ratio

$$\frac{dc}{dt} = \frac{0{\cdot}0008}{0{\cdot}0011} = \frac{1}{1{\cdot}37}.$$

Therefore, the therapeutic coefficient is not favourable.

The action of the preparation on trypanosomes is very energetic.

We have observed the disappearance of trypanosomes in one mouse.

Mouse No. 440—2. iv. 1915, number of trypanosomes ++++; 1 c.c. of a 1 : 1200 solution of tartar emetic was injected intravenously.

5 minutes later the number of trypanosomes					=	+++
10	,,	,,	,,	,,	=	++
15	,,	,,	,,	,,	=	++
20	,,	,,	,,	,,	=	+
25	,,	,,	,,	,,	=	+ few
30	,,	,,	,,	,,	=	+ few
35	,,	,,	,,	,,		
40	,,	,,	,,	,,		
50	,,	,,	,,	,,		
60	,,	,,	,,	,,	=	+ very few
70	,,	,,	,,	,,		
80	,,	,,	,,	,,		
90	,,	,,	,,	,,		
100	,,	,,	,,	,,	=	0

Experiments with Trypan-blue. This preparation was originally introduced in the therapy of experimental trypanosomic diseases by Mesnil and M. Nicolle. In the practical treatment of trypanosomiases it has not been applied, but is very important in the treatment of piroplasmoses as first demonstrated by Nuttall and Hadwen.

The toxicity of the preparation at our disposal is shown in Table 17. Thus, the dosis tolerata is 1 c.c. of the solution 1 : 300.

Table 17.

Solution	Results
1 : 200	Succumbed
1 : 300	Recovered
1 : 400	
1 : 500	

The effect of trypan-blue on the trypanosomes is shown in Table 18.

Table 18.

	Days							
Solution	1	2	3	4	5	6	7	8
1 : 300	++⊕	+++	++++	+++	++++	death		
1 : 400	+v.f.⊕	+	++	+++	++++	++++	++++	death
1 : 500	++⊕	+++	+++	+++	death			
1 : 600	+v.f.⊕	+f.	++	+	++	+++	+++	death
1 : 800	+v.f.⊕	+	+	++	death			
1 : 1000	+v.f.⊕	+	++	+++	,,			
1 : 1500	+⊕	++	+++	+++	,,			
1 : 2000	+f.⊕	++	++	+++	,,			

It is clear that, in this infection, trypan-blue is unsuitable as a trypanocide.

Experiments with trypanil. In our experiments this compound was always administered intravenously and its toxicity is shown in Table 19.

Table 19.

Solution	Results
1 : 200	Succumbed
1 : 300	Recovered
1 : 400	Recovered
1 : 500	Recovered

Thus the dosis tolerata is the same as in the case of trypan-blue, 1 : 200.

The trypanocidal action is shown in Table 20. In addition the preparation was injected subcutaneously and the mouse relapsed after 13 days (Table 21).

Table 20.

	Days										
Solution	1	2	3	4	5	6	7	8	9	10	11
1 : 200	+v.f.⊕	0	0	0	0	0	0	0	0	recurrence	
1 : 300	+v.f.⊕	+f.	+	+++	+++	++	++	++	death		
1 : 400	+v.f ⊕	+v.f.	+	—	+++	++	++	++	++	++	
1 : 500	+v.f.⊕	+	++	+++	death	++	++	++	+++	++	death

Table 21.

	Days			
Solution	1	2	3—12	13
1 : 100	+v.f.⊕	+v.f.	0	recurrence

Experiments with trixidin. This preparation consists of a 30 % oil emulsion of antimony trioxide. For the estimation of its toxicity we have injected subcutaneously from 0·1 to 0·3 grammes of trixidin per 20 grammes of live weight.

The results were as follows (Table 22):

Table 22.

Dose of in grammes	Results
0·1	Recovered
0·15	Recovered
0·2	Recovered
0·25	Recovered
0·3	Succumbed on 7th day

Mice infected with this trypanosome were injected with various doses of this compound (subcutaneously in Province oil) from 0·0005 to 0·1 grammes per 20 grammes of weight of the mouse, which corresponds to a solution of the preparation from 1 : 2000 to 1 : 100.

The results obtained were as follows (Table 23):

Table 23.

Solution	Dose in grammes per 20 gr. weight	Results
1 : 2000	0·0005	Not cured
1 : 1500	0·00066	,,
1 : 1200	0·0008	,,
1 : 1000	0·001	Cured
1 : 800	0·00125	,,
1 : 500	0·002	,,
1 : 250	0·004	,,
1 : 100	0·01	,,

The same is seen with fuller details in the next table (Table 24).

Table 24.

No. of mice	Days 1	2	3	4	5	6	7	Solution of
458	+f.⊕	+ +	+ +	+ +	+ + +	+ + +	death	1 : 2000
463	+⊕	+ +	+ +	+ + +	+ +	+ +	,,	1 : 1500
518	+ +⊕	+ +	+ + +	death	—	—	—	1 : 1200
453	+⊕	+ +	+ +	0	examination during 48 days. No relapses			1 : 1000
455	+⊕	+ +	+	0	examination during 48 days. No relapses			1 : 800
456	+f.⊕	+ +	+f.	0	examination during 46 days. No relapses			1 : 500
459	+f.⊕	+ +	+ +	0		recovery		1 : 250
457	+⊕	+ +	+	—		,,		1 : 100

Thus, we see that the dose of 0·001 gm. per 20 gm. weight of the mouse is sufficient to cure it of trypanosomiasis.

Curing by stages. Under the name of "curing by stages" we mean the introduction of one and the same dose, or different doses of the preparation, with certain intervals of time between the successive injections. Tartar emetic was the first preparation to be tested, as a single injection of this drug cures mice only when introduced in a 1 : 1200 solution, this dose being close to the toxic.

In the first series of experiments (mouse No. 306) a 1 : 5000 solution was injected, and after 2 days 1 : 5000 again. The animal relapsed.

In the second series (mouse No. 324) we made two injections of 1 : 5000 solution, and two of 1 : 7000 (for intervals see Table 25). The animal relapsed.

In the third series (mouse No. 332) we injected a 1 : 5000 solution four times (for intervals see table). There was recurrence.

Thus, curing by stages with potassium antimony tartrate in small doses did not give any positive results.

Atoxyl injected intravenously 2 days in succession in a 1 : 300 solution has driven the trypanosomes out of blood, but 9 days later there was a relapse.

This preparation introduced in the same solution 3 days in succession, and also intravenously, cures the animal.

Table 25.

No. of mice	Days 1	2	3	4	5	6	7	8	9	10	After last injection
306	1:5000		1:5000								
	+v.f.	0	0	0	0	0	0	0	0	recurrence	
324	1:5000				1:7000	1:7000					
	+f.	0	0	0	0	0	0	0	0	0	recurrence on the 26th day
332	1:5000				1:5000		1:5000				
	+f.	0	0	0	0	0	0	0	0	0	recurrence on the 16th day

Table 26.

No. of mice	Days 1	2	3	4	5	6	7	8	9	10	11
534	1:300	1:300									
	+f.	+f.	0	0	0	0	0	0	0	0	+
535	1:300	1:300	1:300	examination during 35 days. No recurrence							
	+v.f.	+v.f.	0								

Combined treatment. In his article, "Chemo-therapy in Veterinary Medicine," Yakimoff refers to the theoretical conceptions which serve as a basis for treatment by combinations of different preparations as established by Ehrlich and Laveran:

(1) Combined treatment produces a greater effect on the parasites, as the action of the drugs increases with regard to the parasites, whilst it does not increase with regard to the organs, provided, of course, that the preparations are carefully chosen.

(2) Only quite tolerable doses of each drug are administered.

(3) Each of the preparations affects the parasites, which are resistant (naturally or by habituation) against one of the preparations. Preparations belonging to different chemical groups should be combined, and those belonging to one group should be of different degrees of resistance.

(4) Methods of administration should be as varied as possible.

We have carried out several such experiments with different preparations, choosing them from different groups (from the arsenic and benzidine groups).

(1) Tartar emetic + trypan-blue
(2) „ „ + trypanil
(3) Atoxyl + trypan-blue
(4) „ + trypanil

} Series A.

Further we passed from this simple *combined* treatment to *combined treatment by stages* on the following lines:

(5) Tartar emetic + trypan-blue + tartar emetic + trypan-blue
(6) „ „ + trypanil + „ „ + trypanil
(7) Atoxyl + trypan-blue + atoxyl + trypan-blue
(8) „ + trypanil + „ + trypanil

} Series B.

The results of our experiments are summarised in Tables 27 and 28.

Table 27. Series A.

No. of mice	Days 1	2	3	4	5	6	7	8	9	10
305	E. 1:5000		T. 1:400							
	+f.	+v.f.	0	0	0	0	recurrence			
302	E. 1:5000			D. 1:400						
	+v.f.	0	0	0	0	0	0	0	recurrence	
301	A. 1:300		T. 1:400							
	+v.f.	+	+	0	0	recurrence				
303	A. 1:300		D. 1:400							
	+v.f.	+v.f.	+v.f.	0	0	0	0	0	0	recurrence

Table 28. Series B.

No. of mice	Days 1	2	3	4	5	6	7	After last injection
329	E. 1:5000	T. 1:400	E. 1:5000	T. 1:400				
	+	0	0	0	0	0	0	recurrence after 20 days
368	E. 1:5000	D. 1:400		E. 1:5000	D. 1:400			
	+	+	+	+	0	0	0	recurrence after 6 days
420	A. 1:300	T. 1:400			A. 1:300		T. 1:400	
	+v.f.	+v.f.	0	0	0	0	0	recurrence after 11 days
370	A. 1:300	D. 1:400		A. 1:300	D. 1:400			
	+v.f.	0	0	0	0	0	0	recurrence after 26 days
462	A. 1:300	D. 1:400		A. 1:300	D. 1:400			
	+v.f.	+f.	+f.	+	+	0	0	recurrence after 6 days

E. tartar emetic; A. atoxyl; T. trypan-blue; D. trypanil (dianil-blue).

Thus, combined treatment with tartar emetic, or atoxyl, and trypan-blue or trypanil, did not produce positive results.

Neither did combined treatment by stages produce any good results.

Preventive treatment. This treatment has been applied in two ways:

(*a*) At first the preparation was injected, and then simultaneously, or after a certain interval, the animal was infected.

(*b*) The animal was at first infected, and then simultaneously or after a certain interval the preparation was injected.

Infection was made subcutaneously, and the following preparations were introduced: tartar emetic, intravenously, and trixidin, subcutaneously. The dose of tartar emetic was 1 : 1200 solution, and of trixidin 1 : 500 and 1 : 250. The results were unsatisfactory. We are continuing the experiments on chemo-therapy.

Serum. Besides chemical preparations, we have also tested different kinds of serum: (1) human, (2) from rabbit infected with the camel trypanosome and (3) anti-anthrax serum (from horse).

(1) *Human serum.* Serum was obtained from a healthy woman. It was applied both for curative and preventive purposes.

Table 29.

No. of mouse	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
185	+⊕	0	0	0	0	0	0	0	0	0	0	+	+ +	+ +	+ +	+ +	death	Human serum
														+ +	+ +	+ +		
28	Infection + serum	Observation during 48 days. No infection																
192	+⊕	+ +	+ +	+ +	+ + +	+ +	death											Serum from trypanosomic rabbit
						+ +												
193	+⊕	+ +	+ + +	+ +	death													Anti-anthrax serum
				+ +														

In the first case (mouse No. 185) the treatment expelled the trypanosomes for a certain time, but later (in 11 days) there was a relapse and the animal succumbed.

In the second case (mouse No. 28), after the injection into different parts of the body of mixed serum and trypanosomic blood, the animal appeared to be quite immune from infection (observation during 42 days).

(2) and (3) *Serum from trypanosomic rabbit and anti-anthrax serum.* These sera not only failed to cure the mouse, but also there was no temporary expulsion of the trypanosomes from the blood.

EXPLANATION OF PLATES III—V.

PLATE III.

Figs. 1 and 2. Trypanosomes in the blood of camel. In fig. 2—dividing form.
Fig. 3. Trypanosomes of camels, passed through mouse.
Fig. 4. „ „ „ rat.
Fig. 5. „ „ „ horse.
Fig. 6. „ „ „ guinea-pig.
Fig. 7. „ „ „ calf (♂).
Fig. 8. „ „ „ pig (juv.).
Fig. 9. „ „ „ ram.
Figs. 10 and 11. Dividing forms. In fig. 11 the trypanosome on the left shows the blepharoplast beginning to divide, in the right one the blepharoplast has already divided. In fig. 12 the blepharoplast and nucleus have divided.

PLATE IV.

Fig. 12. Trypanosome of camels passed through dog.
Fig. 13. „ „ „ rabbit.
Fig. 14. Involution forms in the lungs of mouse.
Fig. 15. *Trypanosoma hippicum* (Murrina).
Fig. 16. *T. soudanense* (El debab).
Fig. 17. *T. evansi* (Surra).
Fig. 18. Agglomeration of trypanosomes from Turkestan camels.

PLATE V.

Fig. 19. Foal infected with trypanosomes. Shortly before death.
Fig. 20. Dog infected by trypanosomes. Shortly before death.

HIBERNATION OF FLIES IN A LINCOLNSHIRE HOUSE.

By G. S. GRAHAM-SMITH, M.D.

In the autumn of 1917 the writer received several letters from Mr L. C. Peters describing his experiences with flies hibernating in a large and very old house, situated in a large park at Frampton, near Kirton, Boston, Lincolnshire. Mr Peters' attention was directed first to the matter towards the end of October. On removing the window sashes the frames were found "black with flies." After fumigation a bucketful of dead flies was removed from a single window frame, and about six bucketfuls from other infested windows. Only one side of the house was affected, but on that side the flies were found in the window frames on all three stories. "Ladybirds" were also present in large numbers.

Early in November the window frames were fumigated with HCN, and all the joints and cracks were syringed first with carbon bisulphide and then with a saturated solution of crude naphthaline in methylated spirit. For a few days no flies were seen, but in the beginning of December they were "as numerous as ever."

A second fumigation with HCN and treatment with carbon bisulphide of the situations which could not be fumigated took place in December. On examining the frames in January no living flies were found in the frames.

The gardener informed Mr Peters that the trouble had recurred to his knowledge every winter for 24 years. It usually began towards the end of July, and the flies were numerous on the windows of the house during the autumn and winter. They disappeared about April as suddenly as they appeared.

Some specimens collected on December 6 and forwarded to the writer belonged to the following species.

	Males	Females
Diptera:		
Musca corvina	7	10
Pollenia rudis	4	5
Pyrellia eriophthalma		2
Limnophora septemnotata		31
Culex pipiens		11
Chalcids:		
Stenomalus muscarum		20
Pteromalus deplanatus		1

The most numerous and troublesome fly was *M. corvina*. They might be present in hundreds on one day and have disappeared on the next. *L. septemnotata* occurred mainly in certain rooms, the outer walls of which were covered with ivy. Rooms infested with large numbers of one of these species seldom contained many of the other.

Mr J. Waterston, who examined the chalcids, writes that "there is no doubt that the bulk are *Stenomalus muscarum*. The dark specimen is a rather undersized example of *Pteromalus deplanatus* Nees (1834)." Mr Grimshaw very kindly identified the "small flies" as *L. septemnotata* Ztt.

On examining an unoccupied house near Cambridge on March 5, 1918, hundreds of living and dead specimens of *P. rudis* were found in several rooms facing west.

BILHARZIASIS IN NATAL.

By F. G. CAWSTON, M.D. (Cantab.).

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INTRODUCTION.

In the following pages I shall endeavour to describe the present state of our knowledge of Bilharziasis in Natal and the result of some personal observations extending over the last seven years. The investigations of the Bilharzia Mission to Egypt[1] have drawn attention to the various forms of cercariae which infest several species of fresh-water snail in African rivers. The cercariae which give rise to Bilharzia disease in Egypt are furcocercous cercariae, *i.e.* they possess a tail which is divided for a greater or shorter portion of its length. They are characterised, further, by the absence of a pharynx at the commencement of the oesophagus. Similar cercariae are present in one of the species of fresh-water snail which abounds in the infected areas of Natal.

In some localities, the custom of the South African natives in anointing the skin with grease and oil may be nature's own preventative against the skin-infection through cercariae. During the visit of the Bilharzia Mission to Egypt it was observed that cercariae would attack all parts of the bodies and limbs of young rats and mice that were immersed in water containing them.

As long ago as 1872, T. S. Cobbold was of opinion that the disease was caused as a result of drinking water containing snails or the little cercariae which are often present in snails from the rushes of slow-running streams or stagnant bathing-pools; but only recently has it been known that infected snails were so intimately associated with the disease.

[1] *Journ. Roy. Army Med. Corps* for 1915—July, August and September.

I have confined myself to the disease as met with in Natal, where it would seem that it is contracted in every instance from bathing in infected rivers and stagnant pools. Since the danger of bathing in these places has become known, and since the municipalities have filtered or strained their town water-supply, the disease has become very much less frequent. I have treated cases from Rhodesia which have presented the same symptoms as those with which we are familiar in Natal.

Treatment consists in the avoidance of re-infection from fresh-water bathing-places and in combating the complications that arise in the severer cases. The disease as generally met with is not a very serious one and its interest lies largely in its relation to Life Insurance.

HISTORY.

Bilharziasis has existed in Africa for many centuries. Specimens preserved from the XIIth dynasty, which is estimated from 2500 to 4000 years before Christ, are said to prove its existence at that period. The cause of the disease was not known until 1852, when T. Bilharz published the report of his discovery of a bisexual distome and successfully established a definite relationship between this trematode worm and the symptoms of dysentery and haematuria, which result from its presence in the human subject[1]. In 1864, J. Harley reported that the endemic haematuria, common to certain parts of Natal and the Cape of Good Hope, was also due to a species of *Bilharzia*[2]. In 1871 he published his attempts to obtain direct infection by the mouth in two young rabbits and two dogs; but no trace of *Bilharzia* could be found[3]. In 1872 T. S. Cobbold published an article on "The Development of Bilharzia haematobia," showing how he had endeavoured to trace the life-cycle through an invertebrate intermediary host[4]. He failed to induce the ciliated embryos to enter the bodies of different species of *Planorbis* and other fresh-water molluscs, but the experiments were undertaken in England. In 1888, Jas F. Allen, of Pietermaritzburg, reported "nearly all the youths bathing in the Umsindusi and Dorp spruit were infected; whilst the girls, who do not bathe, remained free from the disease[5]." This view of infection by bathing is commonly held by all general practitioners in Natal with any experience of the disease.

In a popular lecture to the Rhodesia Scientific Association in Jan. 1914, Sir Patrick Manson said, after briefly outlining the development of the liver-fluke, "In a similar way I believe the germ of *Bilharzia* disease, so common in this country, especially in young people, and probably contracted whilst bathing in pools and rivers, is acquired and spread."

[1] *Zeitschr. f. wiss. Zool.* IV. pp. 53, 72–76, 454.
[2] *Med.-Chir. Trans.* London, XLVII. pp. 55–72, 2 pls.
[3] *Ibid.* LIV. pp. 47–62, 1 pl.
[4] *Brit. Med. Journ.* II. pp. 89–92.
[5] *Practitioner*, XL. pp. 310–320.

In the *Tropical Diseases Bulletin*, March 30, 1914, Fleet Surgeon Kumagawa comments on the publication of K. Mirairi and M. Tuzuki[1]. "The authors noticed that, when ox faeces which contain the eggs of *Schistosomum japonicum* are kept for one or two hours in a suitable temperature, mixed with water, the majority of the miracidia come out, breaking the shell and swimming about very vivaciously. They noticed also that, in the infected locality, there are many snails in the waterways and ditches." The authors carefully picked up a number of young non-infected snails and tried whether the miracidia entered their bodies or not. They found that the miracidia entered the body of the snail. After twelve days the first rediae appeared and gradually concentrated to the hepatic ducts. The authors put mice into the vessels in which the full-grown snails were fed for three hours every day, and repeated this experiment for four days. After three weeks they found a great many *Schistosomum japonicum* in the livers of the mice. The authors concluded that this kind of snail was an intermediary host of *Schistosomum japonicum*. These observations were confirmed by the Wandsworth Expedition of the London School of Tropical Medicine, in the spring of 1914.

EXPERIMENTS IN NATAL.

In May 1915 I made repeated attempts to infect specimens of *Limnaea natalensis* with miracidia obtained from recently passed urine. There was practically no possibility of these fresh-water organisms being infected with *Bilharzia* when the experiments were commenced, nor could any evidence of infection be detected at the end of several weeks. These experiments were repeated in September, but again with negative results. From further study I am of opinion that *Limnaea natalensis* is not susceptible to infection with the miracidia.

Judging from the reproduction of digenetic trematodes, Dr E. Warren, the Director of the Natal Government Museum, was of opinion that the miracidia had the asexual phase of its development in the mollusca; that it would die if unable to harbour in a suitable mollusc in 24 to 48 hours and that, if it was able to migrate to the liver of a mollusc, the sporocyst—a smooth-walled elongated sac—would develop in the course of a week or two. He expected to find the sporocyst giving rise by budding of its wall to daughter-sporocysts and to contain a large number of cercariae with bifid tails.

At the end of May 1915, I supplied him with urine obtained from a patient suffering from Bilharziasis. He diluted this urine, which swarmed with ova, and added it to a vessel of water containing snails, *Physopsis africana*. The bathing season had not commenced and the snails were probably free from infection when the experiments began. Four weeks later, he wrote me a letter in which he said: "I have found some undoubted cercariae in infected snails, and the question now is whether they belong to *Bilharzia* or to some

[1] *Tokio Med. Journ.* Sept. 1913.

other trematode. Unfortunately, it is quite possible that the sporocysts and cercariae found are common in the snails and that the urine added to the water did not really infect them." In 1916, during the months of February to June, I collected and examined 500 specimens of this genus of fresh-water snail from various parts of Natal. Some of these were infested with cercariae; but I found cercariae, typical of *Bilharzia* infection, possessing a divided tail and showing no muscular pharynx, only in specimens from two bathing-places which are known to be associated with Bilharziasis amongst swimmers.

The sporocyst which contained the cercariae which Dr Warren found in the specimens of snail he had exposed to *Bilharzia* infection was an elongated body without oral sucker or alimentary canal. Through its transparent walls could be seen a large number of cercariae in various stages of development. When teased out and stained, the cercariae were seen to consist of a body and a tail. The body possessed a terminal oral and a ventral sucker. There were no pigment spots and no muscular pharynx. The tail was bifid. There was no cuticular keel along either side of the prongs of the tail.

In a stagnant pool near Durban which is known to give rise to Bilharziasis amongst bathers, I have found 15 sporocysts answering to this description and containing similar cercariae, in specimens of this same snail.

The appearance of both sporocyst and cercariae corresponded to those described by Leiper and Atkinson[1] as characteristic of Asiatic schistosomiasis.

RECENT RESEARCHES.

The Bilharzia Mission to Egypt, according to a full report by Lt-Col. Leiper[2], demonstrated that the parasitic worms which gave rise to Bilharziasis in Egypt developed from the cercariae which infested certain species of fresh-water snails. The Mission found that infection might take place orally or cutaneously. The report is illustrated, and one micrograph shows the cercariae in the act of passing through the unbroken skin of a newly-born mouse. Later, Lt-Col. Leiper[3] reported that those cercariae which infest specimens of *Bullinus* (sic) invariably develop into *Schistosomum haematobium*; whilst the cercariae which infest the *Planorbinae* of Egypt always produce *Schistosomum mansoni*.

DISTRIBUTION.

Practically all the rivers between Pietermaritzburg and the coast are said to be infected with *Bilharzia*, though it would seem that the disease is not to be caught at higher altitudes in Natal. During a somewhat extended tour of Natal in the spring of 1916, I visited the following places to ascertain the prevalence of susceptible fresh-water snails in infected areas. I found specimens of *Physopsis africana* difficult to obtain in any places except where the water was stagnant or slow-running.

[1] *Brit. Med. Journ.* Jan. 30, 1915.
[2] *Journ. Roy. Army Med. Corps*, 1915.
[3] *Brit. Med. Journ.* March 18, 1916.

Date	Place	River	Observations
Feb. 14	Verulam	Umhloti	Reeds clear after rains
,, 16	Avoca	Little Umhlanga	*Physopsis* plentiful in infected pools
,, 16	Toll Gate (Durban)	Brickfields	*Physopsis, Planorbis, Limnaea* and *Isidore* plentiful in infected pools
,, 17	Hill Crest	Umhlatazana	Reeds clear in river, bathing not allowed
,, 18	Umlaas Bridge	Umlaas	*Physopsis* plentiful in infected river
	Maritzburg	Quarry	Only *Limnaea*, doubtful infection
Mar. 10	,,	Boshoff St. pool	Bathing forbidden. No *Bilharzia* cercariae. *Physopsis, Limnaea, Planorbinae* and *Ancyli* plentiful
,, 31	,,	Umsindusi	*Physopsis* and *Limnaea* plentiful. *Bilharzia* cercariae in *Physopsis*. Infected river
	Greytown	River	Reeds clear and no infection known
Feb. 16	Durban	Umgeni	Reeds clear
May 1	,,	,,	*Physopsis* and *Planorbis*. An infected place
,, 13	Henley	Umsindusi (near source)	Reeds clear in river. No infection known
June 23	Maritzburg	Brickfields	Reeds clear. No bathing

THE FRESH-WATER SNAILS OF NATAL.

I have encountered the following species of snail in Natal rivers and fresh-water pools:

Limnaea natalensis is a common light brown snail with a semi-transparent dextral shell. Some specimens are infested with "tadpole" cercariae.

Physopsis africana is a common black snail with a blunt-pointed sinistral shell, the shell is characterised by a truncate columella. This snail harbours both the "tadpole" and furcocercous cercariae; but *I have only found the latter forms of cercariae in bathing-places which are known to give rise to Bilharziasis.*

Planorbis pfeifferi is similar to the intermediary host of *S. mansoni* in Egypt. It is a common brown snail with a round, flat shell.

Planorbis leucocheilus is a light brown, less common snail. The shell is small and flat. *Planorbis pfeifferi* harbours many "tadpole" cercariae.

Isidora tropica is a fairly common, dark brown snail with a blunt-pointed, sinistral shell. I have never found cercariae in this species.

Isidora forskali is a rare, brown snail with a conical shell, I have only seen about three specimens of it.

In one brickfield, I found a large number of a small oval snail; which is a new species of *Ancylus* not yet identified.

The following table shows the percentage of snails harbouring *Bilharzia* cercariae between the months of April and July, 1916.

Month	Source	No. infected	Percentage	Form of cercaria
April	Toll Gate (Durban) (bathers infected)	1 out of 7	14	human
May	,, ,, ,, ,,	13 ,, 85	15	12 human (1 eye-spotted)
June	,, ,, ,, ,,	2 ,, 13	15	human
July	,, ,, ,, ,,	8 ,, 131	6	,,
April	Umsindusi (bathing not allowed)	30 ,, 197	15	1 or 2 human
May	,, ,, ,, ,,	38 ,, 170	22	,,
June	,, ,, ,, ,,	7 ,, 30	23	almost all *C. secobii*
July	,, ,, ,, ,,	0 ,, 6	0	
June	Boshoff St. (no bathing)	0 ,, 20	0	
July	,, ,, ,,	0 ,, 6	0	

FURTHER EXPERIMENTS IN NATAL.

In the spring of 1916, I made an exhaustive study of the commoner fresh-water snails of Natal. I examined, microscopically, the livers of 1500 specimens. They included seven different species of snail. I could find no *Bilharzia* cercariae in any species of snail except in *Physopsis africana*. I examined 250 *Planorbinae*. It is interesting to note that *Schistosomum mansoni* which has this species of snail in Egypt as its intermediary host is unknown in Natal. I encountered three different forms of *Bilharzia* cercariae in the specimens of *Physopsis africana* which I examined, one was an eye-spotted form, for which I have suggested the name of *Cercaria bilharziella lunata*, in view of the crescentic form of its eye-spots. Another, for which I have suggested the name of *Cercaria secobii*, has very long prongs to its divided tail; the third form corresponds to that which causes Schistosomiasis in Egypt and the Far East. In one bathing-place which is known to be a source of Bilharziasis, I found 14 % of the specimens of *Physopsis africana* infected with this human form of cercaria. In the April number of the *Medical Journal of South Africa*, Dr J. G. Becker reported that he had found similar cercariae in three out of 13 specimens of this same snail which he had collected from an infected bathing-pool at Nijlstroom in the Transvaal.

I then undertook experiments to infect with miracidia, obtained from the urine of *Bilharzia* patients, a number of this species of snail.

On April 22, I found that 14 out of 31 specimens, obtained from the Umsindusi river, which I had exposed to infection by miracidia three weeks previously, contained *Bilharzia* cercariae—*i.e.* 45 %. Examination of 197 specimens direct from the river showed an infection of only 15 %.

During May, when 22 % of the specimens in the river were infested with *Bilharzia* forms, the percentage of infected snails amongst 19 which had been exposed to infection by miracidia 36 days previously was 37 %.

On June 9th, 60 specimens from the Umsindusi which had been exposed to infection on May 20 were examined for cercariae. Sixteen contained *Bilharzia* forms. Of 30 specimens from the same source, which had been kept for three weeks in clean water, seven were infected.

Similar experiments with *Planorbinae* and *Limnaea* proved entirely negative.

Whilst these experiments were in progress, I attempted to produce *Bilharzia* infection in 12 white rats, six guinea-pigs and six pigeons. During 1915, Dr E. Warren had undertaken similar experiments, submitting rabbits and mice to hypodermic infection with *Bilharzia* cercariae, but with negative results.

In my experiments, I immersed rats and guinea-pigs for an hour on several days in water swarming with *Bilharzia* cercariae; to others, I gave water containing cercariae by the mouth or rectum. No symptoms were produced at the end of three months and no parasitic worms were discovered post-mortem.

I am not aware that Bilharziasis has been demonstrated in any animal in South Africa, except in man. Dr Leipoldt, the Medical Inspector of Schools in the Transvaal, tells me that he has not succeeded in infecting guinea-pigs. However, he is certain that the house-fly carries the egg from the urine and that it can infect monkeys in this way, but, in view of the recent observations reported from Egypt on the life history of both forms of the parasite, this opinion would seem to be of little importance.

PROGNOSIS.

The duration of the disease is extremely variable. In the milder cases there is seldom any pain and the slight haematuria is not noticeable for more than a few weeks. In others, the disease runs a very protracted course, extending over many years. Many cases recover and show no after-effects from the disease, but sterility is common amongst patients who have suffered severely in their younger days. Even in its severest forms, Bilharziasis as met with in Natal seldom proves fatal; although, when complicated by an attack of cystitis or renal stone, fatal symptoms may and do occur. Favourable symptoms are—an absence of anaemia and renal colic, an absence of mucus in the urine and only slight and occasional haematuria. Renal colic is common and does not necessarily point to the presence of renal calculus; it is almost certainly due to the accumulation of ova in the kidney substance. These ova have become loose in the blood-stream and have not made their way up the ureters from the bladder, as popularly supposed.

The occasional presence in the urine of blood and mucus, or of shreds of mucous membrane, indicates the need for urinary antiseptics and diuretics, if permanent damage to the bladder-wall is to be avoided. Microscopic examination of the urine will often reveal the presence of ova, even though the absence of symptoms of the disease for several years had indicated that the patient was cured. In one Government School in the Transvaal, Rustenburg, between 30 and 40 % of the boys showed symptoms of Bilharziasis; microscopic examination of the urine revealed the fact that 76 % of the boys were infected. It is probable that the eggs will continue to escape from the body for some months after the worms themselves are dead. Occasional aggravation of symptoms in patients indicates that they continue to harbour parasites in the blood-stream, even though they have not exposed themselves to fresh infection for many years.

LIFE INSURANCE.

In course of conversation with the Resident Manager for Natal of the National Mutual Life Association of Australasia, he stated that the Company would not accept a case of Bilharziasis so long as the disease was sufficient to produce albumen in the urine. This attitude is a commendable one, for one could not say for certain whether the presence of albumen was entirely due to the disease of itself. But this statement was of especial interest in view of

the fact that I had recently advised a further examination of an applicant whose urine contained a slight trace of albumen. Later, I heard that the applicant had suffered from Bilharziasis, and a subsequent examination of the urine revealed the presence of ova containing miracidia. The Manager stated, further, that the Company would accept at the usual rates for Life Insurance persons with a past history of the disease whose urine was free from albumen.

The Manager for Natal of the Southern Life Association did not consider a history of Bilharziasis in the past very unfavourable, but he drew attention to the risk of gonorrhoeal infection in patients with a past history of the disease.

If an applicant for Life Insurance had noticed no symptoms of the disease for the last twelve months and occasional examination of the urine during the last six months had shown no presence of ova or other abnormality, I should recommend a Company to accept the case without a loading.

If examination of the urine revealed the presence of ova, I should advise a fresh examination in three months and not advise that the case be accepted until the urine had been clear for six months.

If renal colic had been severe and frequent, albuminuria present, a low specific gravity of the urine which is a not uncommon after-effect of the disease, or other sign of permanent kidney mischief, I would naturally advise refusal of the applicant.

The following opinions of Natal practitioners given in 1893 and 1906 in regard to the seriousness with which Bilharziasis should be considered in its relation to Life Insurance is instructive, and it is interesting to note how little they altered their views, after further experience of the effects of the disease. In one case, only the opinion expressed in 1893 can be quoted:

1. Have you had any death attributable to Bilharziasis?

Dr. A. 1893 (after eight years practice in Natal and Cape). No.

Dr B. 1893 (after ten years practice in Natal). No.
1906 (after twenty-three years practice). From secondary effects, but extremely rare; I know of two cases only.

Dr C. 1893 (after ten years practice in Natal). No.
1906 (after twenty-three years practice). No.

Dr D. 1893 (after ten years practice in Natal). No.
1906 (after twenty-three years practice). No.

Dr E. 1893 (after ten years practice in Natal). Yes, indirectly. One case at P.M.: examination showed kidneys congested and deposits of gravel. Patient had suffered from gravel, albuminuria and renal colic. Ova were numerous and I attributed death to Bilharzia.
1906 (after twenty-three years practice). Not directly.

2. Have you had life shortened by this affection?

Dr A. 1893. In one case only, which I attributed to chronic cystitis, due to Bilharzia. Cystitis began at age of 10 years. Death occurred with convulsions at age of 16 years. P.M. examination showed much inflammation of the bladder, but no examination for Bilharzia was made at time of death, nor for two or three years before then.

Dr B. 1893. No.

1906. Not apart from two cases mentioned.

Dr C. 1893. No.

1906. No. The most serious case I have seen, with intense anaemia, got better in time with no treatment.

Dr D. 1893. Cannot say.

1906. I should say no.

Dr E. 1893. Yes, but very rarely.

1906. I think so, but only very occasionally.

3. Have you observed any serious symptoms attributable to Bilharzia?

Dr A. 1893. With exception noted above, no.

Dr B. 1893. Yes, I have formed the opinion that occasionally I have had symptoms in themselves serious, which I think may be attributed to Bilharzia if the theory of the ova forming nuclei for gravel be correct.

1906. I believe renal colic is not infrequent in lads who have had Bilharzia.

Dr C. 1893. Yes. Anaemia after constant drain from the bladder. I have only had one such case out of, say, 100 that have been under my treatment. If the theory of gravel arising from ova of Bilharzia be correct, and I think it is, from the fact of so many gravel cases having a history of Bilharzia, then I have had severe symptoms indirectly.

1906. Anaemia is common but not serious, and only in aggravated cases and the young have I had slight albuminuria which passes off.

Calculi. It is common to find those suffering from above to have at one time had Bilharzia.

Dr D. 1893. Can recollect two cases in which general health suffered consequent on Bilharzia.

1906. I have had cases where for a time the general health has been impaired but those have been patients grown up when they had the disease.

Dr C. 1893. Yes. Not infrequently anaemia and renal symptoms. I had two cases aged 10 years and 15 years with chronic dysentery and griping pains suggesting an ulcerated condition of the bowel. These, when examined, showed Bilharzia and I attributed the condition of both to Bilharzia.

1906. Albuminuria rare and only in extreme cases. I have seen very severe haemorrhage in two or three cases, but in each it passed off quickly. Slight haemorrhage is common and occasionally anaemia. Sometimes, but rarely, renal colic from gravel.

4. Have you found any cure for the disease?

Dr A. 1893. None, certain; the disease usually cures itself.

Dr B. 1893. No. I sometimes give Iron and Turpentine and find at least that the patients are none the worse for it.

1906. No. In lads it will probably cure itself about the age of puberty.

Dr C. 1893. No. The disease will probably cure itself in time.

1906. No. Salol, Santonin or tonics may do good. They often lessen bleeding.

Dr D. 1893. No. Nature seems to cure in most cases.

1906. No.

Dr E. 1893. Iron may assist, but they usually grow out of the affection.

1906. No. The disease tends to die out of its own accord.

5. How would the fact of a patient being the subject of Bilharzia affect him in medical examination for Insurance?

(*a*) When the symptoms are slightly marked:

Dr A. 1893. No loading.

Dr B. 1893. Not at all.

1906. If no albuminuria, if symptoms slight, general look healthy, patient, say, 20 years of age, I should pass the life with a light loading of, say, three years to be taken off when the patient is quite better.

Dr C. 1893. Not at all.

1906. If symptoms slight, if patient 23 or 24 years old and if applicant has had the affection two or three years, I take no notice of it.

Dr D. 1893. If on examination of urine, ova are detected, add a few years for the protection of the Company, though you yourself may not think anything of it.

1906. If patient of strong physique, temperate and otherwise in good health, pass at ordinary rates.

Dr E. 1893. In case of three or four years' standing, without serious symptoms and no albuminuria. If ova have disappeared, ordinary rates.

1906. If applicant has been affected a good while and is getting better, I would (other circumstances being favourable) pass him as a first-class life.

(*b*) When the symptoms are well marked:

Dr B. 1893. Add three years or more, according to symptoms.

1906. Would defer the case, but not refuse it.

Dr C. 1893. Load the life three or four years. If anaemia profound or renal colic, refuse the life.

1906. Might load, because of after trouble from calculi.

Dr D. 1893. Would be regulated entirely by circumstances.

1906. Wait for a few months and then re-examine.

Dr E. 1893. Defer till better or slightly marked.

1906. In extreme cases, refuse the life. Moderately severe, without anaemia and albuminuria slight, I would load the life three to five years.

TREATMENT.

The treatment of Bilharziasis in Natal consists in:

(*a*) avoiding fresh infection from the bathing-pools,

(*b*) destroying the parasites which exist in the portal and mesenteric veins. So far we do not know of any effective remedy for this,

(*c*) hastening the exit of the eggs from the bladder,

(*d*) correcting the bacilluria and haematuria,

(*e*) correcting the anaemia,

(*f*) treating the renal colic, gravel and cystitis,

(*g*) surgical remedies to remove calculus or to relieve retention of urine.

The consensus of opinion would seem to be that it is best to resort to the almost exclusive use of diuretics and urinary antiseptics in uncomplicated cases of the disease. The use of hexamine is of undoubted service in many cases and may be combined with small doses of Buchu. Where there is continuous renal colic, Sodium salicylate and Tr. Hyoscyamus are indicated.

Amongst the various remedies which are employed in Natal may be mentioned:

(*a*) Methylene blue, Male fern, Thymol, Santonin and Salol, to destroy the parasites in the blood-stream. Injections of Salvarsan have been tried without effect, and the mercuric-bichloride treatment of Egypt seems to be quite ineffectual in Natal.

(*b*) Buchu, Turpentine, Hexamine and Cystopurin, to accelerate the escape of eggs and to counteract the bacilluria.

(*c*) Adrenalin in severe cases of haematuria.

(*d*) Tr. Hyoscyamus and Sodium salicylate for the renal colic and injections of morphia where required.

(*e*) Iron and tonics for the anaemia.

It should be borne in mind that the disease is intractable and in many cases resists all forms of treatment; whilst it is the opinion of many with a large experience of the disease that where complications do not arise, the disease may be left to die out of itself without treatment of any kind. My own feeling is that, so long as there is haematuria and flakes of mucous membrane in a cloudy urine, the patient is well advised to persist in taking some form of urinary antiseptic; but, until we are in possession of a drug which is known to destroy the parasites, it is unwise to persist in the use of uncertain remedies.

CONCLUSIONS.

1. That *Bilharzia* cercariae, similar to those which have been demonstrated to cause Bilharziasis in Egypt, occur in Natal in specimens of *Physopsis africana*.

2. That specimens of *Physopsis africana* abound in infected localities, and that a large proportion of these are infected with *Bilharzia* cercariae.

3. That specimens of *Physopsis africana*, when exposed to infection from the urine of *Bilharzia* patients, increase in the number of infected forms.

4. That experimental infection of animals has been unsuccessful and, as far as is known, animals in Natal are not susceptible to the disease.

5. That it is rare to come across a person whose life has been shortened or whose death has been caused from Bilharziasis in Natal.

6. That, until some specific is known, palliative measures are the only ones that can be recommended in the treatment of the disease.

7. That municipalities would be well advised to follow the example of Durban in filtering their water-supply, or, where this is impracticable, the water should be strained through such copper ribbons as are in use at Pietermaritzburg which preclude the possibility of infected snails entering the reservoir from which the town supply of water is derived.

8. That, in infected areas, river bathing should be forbidden and suitable swimming-baths provided.

THE CERCARIAE OF THE TRANSVAAL.

By F. G. CAWSTON, M.D. (Cantab.).

During the months of September, October, November and December 1917 I collected 1250 different specimens of fresh-water snails from various parts of the Transvaal, more especially from the sources of the Vaal and Crocodile rivers. All the larger specimens I submitted to microscopic examination for possible infection with cercariae. From the mud at the bottom of the Mooi river at Potchefstroom, which is a tributary of the Vaal, I came across some very finely preserved specimens of *Unio caffer* Krauss, a large number of which contained small pearls attached to the shell. The amount of lime in the bed of this river probably accounted for the perfect state of the umbones of these *Uniones*. Amongst the small stones at the bottom of the river I collected some remarkably large specimens of *Corbicula radiata* Parreyss. The river also contained some specimens of *Pisidium*, a much smaller variety, not yet identified. On the decomposing rushes at the edge of this river there were numerous specimens of *Ancylus*, for which Mr Henry C. Burnup has suggested the name *Ancylus cawstoni*; I have seen similar specimens at Klerksdorp and in the Hex river at Rustenburg, a branch of the Little Crocodile. All these specimens were free from cercarial infection.

In the tributary of the Little Crocodile at Magaliesburg, I collected several specimens of *Limnaea natalensis*. One of these was infested with motile rediae, provided with oral sucker and a pair of lateral locomotor appendages. These rediae contained fully developed leptocercous cercariae with a chain of about twenty minute "cysts" on either side of their divided gut. The life-history of this trematode worm is at present unknown, but the cercaria and redia had the appearance of those I had isolated from *Limnaea natalensis*, *Planorbis pfeifferi* and *Physopsis africana* at Durban and described under the heading *Cercaria catenata* in the (American) *Journal of Parasitology*, March 1917. A specimen of *Physopsis africana* from the same pool at Magaliesburg contained cercariae typical of the Schistosome group developing in a branching sporocyst. The cercariae were not fully developed. The full-sized *Bilharzia* cercaria which I found in specimens of *Physopsis* at Rustenburg was 0·6 mm. in length. At this point on the Hex river, a tributary of the Vaal, 70 % of the boys and 10 % of the girls harbour the Bilharzia parasite. The flat, fin-like prongs of the tail of the Schistosome cercaria are about one-quarter the length of the tail itself. I have found *Physopsis* along the course of the Little Crocodile river at Mulder's drift also infected with this cercaria. At

this place I found a pool heavily infested with *Physopsis* and a few small specimens of *Planorbis* and *Ancylus*.

On December 8th, I visited the Schoonspruit at Klerksdorp, to investigate a recent outbreak of Bilharziasis amongst the lads who swim in this river, and brought back with me 95 specimens of fresh-water snails from the stagnant water. This river flows into the Vaal. A number of these were *Planorbis gibsoni* Nelson, others were *Limnaea natalensis*. This establishes a new locality for *Planorbis gibsoni*. They also included an interesting variety of *Isidora* which does not agree with any of the other forms I have collected elsewhere—*Isidora tropica* from Durban, *Isidora forskali* and *Isidora compta* M. & P. from Maritzburg or the *Isidora* I have collected at Potchefstroom.

There were probably four distinct species of trematode worm represented among the cercariae with which these *Isidora* from the stagnant pools at Klerksdorp were infested. Each of these cercariae was about 0·6 mm. in length; the elliptiform body being about 0·2 mm. by 0·2 mm.; but varying considerably in shape, as the cercaria by means of its two suckers moved along the surface of the glass slide. In each case the long slender tail was very motile, even after becoming detached from the body. Three of the snails contained motile rediae, producing leptocercous cercariae, which were found to be escaping from the liver-substance of the snail. The liver-substance of one of these snails was orange in colour, from the presence of numerous golden granules in the rediae it contained. These granules would appear to exist outside the gut and outside the cercariae produced in the rediae. In each case the redia was provided with an oral sucker, an alimentary canal and two pairs of locomotor appendages. In two of the specimens, the leptocercous cercariae were seen to possess small pharynxes at the commencement of the oesophagus. Another specimen was seen to possess sporocysts producing daughter-sporocysts without the formation of rediae.

CERCARIA ARCUATA.

One of these *Isidora* from the Schoonpoort at Klerksdorp contained rediae with terminal oral sucker and two small lateral locomotor appendages. These contained leptocercous cercariae without pharynx which possessed a chain of cystogenous vesicles on either side of their divided gut. In most of these cercariae, the chains had the appearance of being joined, posterior to the ventral sucker, so that the pair of chains presented the appearance of an inverted horse-shoe. I have suggested the name *Cercaria arcuata* for this variety.

CERCARIA FRONDOSA.

I have collected over 400 specimens of *Isidora* from a stagnant pool at the Golf Course at Potchefstroom. On reference to Jickeli's *Fauna N.-O.-Afr.*, Mr Henry C. Burnup of Maritzburg has no hesitation in identifying these *Isidora* as *Isidora schakoi* Jickeli. This he does in full knowledge that

Connolly has removed *schakoi* from the S. African list, for the shells do agree very well with Jickeli's description and figures of *schakoi*.

Most of the larger specimens of *Isidora schakoi* Jickeli, collected from the Golf Links at Potchefstroom, were found to be infested with an interesting eye-spotted cercaria, *Cercaria frondosa*. This cercaria is about 0·6 mm. in length, the tail being about twice the length of the body. The body is elliptiform and possesses oral and ventral suckers. The body of this ephemera is somewhat heavily coloured, particularly in the fully developed cercaria. This makes the internal organs hard to define. The younger specimens possess a pair of deeply pigmented eye-spots, just posterior to the oral sucker and on either side of the gut. In the older cercariae, which are really beautiful, these eye-spots are seen to branch out in all directions and present the appearance of leaves. In well developed forms this pigment is continued down the sides of the gut, suggesting that it is connected in some way with an elementary nervous system. On either side of the gut, chains of cystogenous vesicles may be seen in some of these cercariae. In some instances these chains are seen to branch. After staining with various stains, the body of about one in five of the cercariae is more darkly stained than the others, and, as this appearance is fairly constant, it is possible that the two forms represent different sexes.

The eye-spots can be seen through the semi-transparent wall of the rediae. These rediae are slightly motile, and possess an oral sucker at one extremity. A varying amount of coloured matter can be seen in the rediae outside the gut. They also possess two very small appendages on either side, not far from the oral extremity.

CERCARIA GLADII.

Two specimens of *Isidora schakoi* from the Golf Links at Potchefstroom contained sporocysts and daughter-sporocysts without the formation of rediae. These sporocysts contained a cercaria with oral and ventral sucker, no pharynx at the commencement of the oesophagus and two flat prongs at the extremity of the tail. The body was 0·2 mm. in length and breadth, the tail without the prongs 0·15 mm. in length and the prongs 0·25 mm. The prongs were not so long as those of *Cercaria secobii* from Pietermaritzburg (see (American) *Journ. of Parasitology*, March 1917), and presented the appearance of drawn swords. These prongs were constantly longer than those of the cercaria which produces Bilharziasis in the Transvaal. The cercaria, for which I have suggested the name, *Cercaria gladii*, probably represents the cercarial stage of the trematode parasite of some of the frogs, crabs, small birds or cows that frequent these parts; though examination of a number of the crabs has failed to throw any light on its possible life-history.

I have collected several specimens of *Limnaea natalensis* from this same pool at Potchefstroom, but have found them free from infection with cercariae. The specimens of *Limnaea natalensis* that I obtained from the Hex river at Rustenburg were the finest I have seen; here, I also obtained some large *Planorbis pfeifferi*, which I have not encountered at Potchefstroom.

CONCLUSION.

It would seem that the specimens of snails found in the flowing rivers are considerably less infected with trematode parasites than those occurring in stagnant pools. The specimens obtained from the river-bed of the Vaal and its tributaries are remarkably well preserved, possibly from the amount of lime in the river-bed. The inference is allowable that the absence of cercarial infection in river snails is also due to the lime; this will also account for a relative infrequency of Bilharziasis along the course of the Vaal river, as compared with those portions of the Low Veldt through which the Crocodile river and its tributaries flow.

THE STRUCTURE OF THE MOUTHPARTS AND MECHANISM OF FEEDING IN *PEDICULUS HUMANUS*.

By Lieut. A. D. PEACOCK, M.Sc., R.A.M.C. (T.).

(With Plate VI and 6 Text-figures.)

In my previous paper on the body louse (1916) many points of morphological interest were necessarily left in abeyance and mention was made that certain statements in it were open to correction. Further work has rendered correction possible. While working in France, to ascertain whether *Pediculus humanus* was a carrier of the spirochaete of Weil's disease, opportunity was taken to investigate those organs of the louse in which the spirochaete might lodge and the afferent and efferent channels through which it might pass. The enquiry did not demonstrate the presence of the spirochaete in the body louse, but incidentally a detailed knowledge of the mechanism of feeding was obtained and is here presented. Since the main conclusions of this paper were reached (December, 1916) I have read Harrison's paper, "A Preliminary Account of the Structure of the Mouth-Parts in the Body-Louse," and while much of the following verifies his statements, we differ on certain important points regarding the structure of the mouth-parts and musculature.

Grateful acknowledgment is made for the assistance rendered by Professor G. H. F. Nuttall, F.R.S., Quick Laboratory, Cambridge, Mr A. W. Bacot, Entomologist to the Lister Institute, Mr Lloyd, Chief Entomologist to N. Rhodesia, Captain J. Shaw Dunn, R.A.M.C., the late Major Sidney Rowland, R.A.M.C. and Captain Adrian Stokes, R.A.M.C.

Professor Nuttall, on my showing him my MS. and preliminary drawings, kindly showed me his preliminary drawings of serial sections of the head of the louse; Mr Bacot lent to me a slide of sections of the same region and, in addition, both gentlemen made helpful suggestions regarding the text and drawings. Mr Lloyd has kindly permitted me to express an opinion on one of his dissections. Captain J. Shaw Dunn's practised technique was responsible for the best series of sections upon which my main conclusions are based, while the late Major Rowland and Captain Stokes generously placed at my disposal some of their own private material and apparatus.

Technique. To obtain adult specimens with the mouth-parts extruded is, in my experience, largely a matter of luck. Hungry lice may be placed on the arm and allowed to feed freely and undisturbed for a few minutes. A few drops of chloroform poured quickly over the insects renders them helpless sufficiently long to enable them to be transferred to 5 % formalin. The

specimens so treated may be examined under the low power to see if the parts are extruded. This method was successful on the first trial, the stabbers being extruded fully, but scores of attempts since have resulted in failure. Occasionally a specimen may be obtained which shows the stabbers partly out. Better results are possible with very young lice. The use of ethylchloride as a killer gave no better success than chloroform.

Only occasionally was a binocular dissecting microscope available and many dissections were made under the $1\frac{1}{2}''$ objective of an ordinary microscope. With a little practice the stabber sac and its organs may be readily freed from the head. To do this three cuts may be made with the edge of a triangularly sharpened dissecting needle, two cuts being sagittal along the lateral chitin of the head and one transversely along the post-dorsal chitin. The dorsal chitin of the head, after scraping beneath it, may be removed like a lid. With a little more dissection the whole sac and its contents may be isolated.

Specimens for section cutting were obtained by fixing in 5 % formalin for one day; dehydrating in alcohol, 50 %, 75 %, 90 %, absolute, twelve hours in each; clearing in xylol, one day at least; placing in a mixture of xylol and paraffin wax (m.p. 54° C.), equal parts, for twelve hours and then in wax for periods ranging from 30 minutes to one day. Sections were cut about 10μ in thickness, using an exceedingly sharp razor and a Cambridge rocking microtome.

The choice of stains only permitted one series of sections being treated with Mayer's haematoxylin, intensifying the blue with ammonia and differentiating with eosin. This method gave the best results and all mention in the text to the colours of stained tissue refers to it[1].

In interpreting sections, two important items should be borne in mind: (1) the shrinkage and distortion of tissue and organs due to treatment, (2) the degree of protrusion or retraction of the mouth-parts.

GENERAL FEATURES OF THE HEAD.

Three regions may be distinguished in the head of the louse when viewed dorsally (Text-fig. III) (1) the anterior bluntly pointed *clypeus*, (2) the central broader, somewhat domed *epicranium*, (3) the posterior, narrower *occiput*. Anteriorly from the clypeus there often projects a small, short, blunt rostrum —the "*haustellum*." The lateral chitin of the clypeus is thickened and frequently pigmented dark brown. The epicranium, just behind and laterally to where it merges with the clypeus, bears the antennae and, at its broadest region, laterally, also bears the eyes. The lateral chitin behind the eyes, as far back as the occiput, is thickened and often pigmented like that of the clypeus. The posterior part of the occiput is sunk into the thorax.

[1] The main conclusions are based on the examination of twenty cleared specimens of heads, a few dissections and a single series of transverse sections cut by Capt. Dunn. Certain details, however, have been worked out from the sections of another sixteen series (eight transverse and eight longitudinal) which had not been so successfully prepared. The gross structure of the parts was drawn with the aid of an Abbé camera lucida.

The relationship of the structures surrounding the mouth, when the louse is not feeding, is best understood by reference to the accompanying illustrations.

The "*haustellum*," in Pl. VI, fig. 1, is shown slightly protruded from the thin chitinous diaphragm which forms the blunt front of the head. It is incompletely circular, the periphery being interrupted ventrally thus forming the *buccal slit* (Text-fig. I). The chitin of the haustellum is continuous with that which lines the entrance to the food canal. Anteriorly are "two *dental plates*, which are everted in the figure and loosely hinged to the buccal plate" (Harrison). This is confirmed in my sagittal sections. From the base of the haustellum, on each side of the buccal slit and at the opening of the mouth, strong chitinous structures festoon posteriorly and laterally to the side of the clypeus; these may be called the *buccal festoons*.

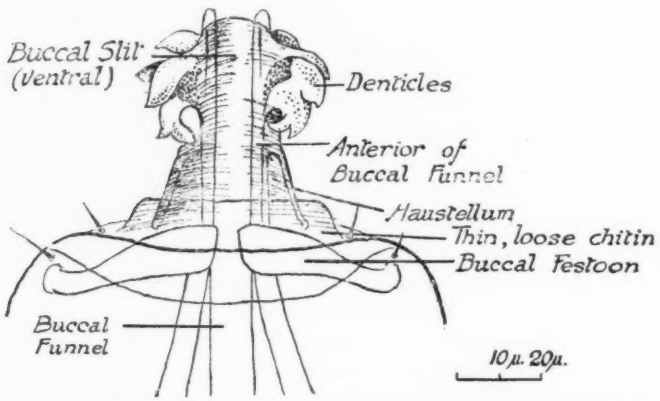

Fig. I. *Pediculus humanus*. Anterior portion of head in dorsal aspect showing the everted denticles. The stabbers omitted.

The general scheme of the internal mechanism concerned in feeding may be understood by reference to Plate VI, fig. 1. The two main features are (1) the *food canal* and (2) the *stabber-sac*. The buccal region consists mainly of the *buccal funnel* (*b.f.*), it leads to the relatively large bladder-like *pumping-pharynx* (*p.p.*), which narrows greatly posteriorly, and opens into the muscular, bulbous *pharynx* (*ph.*), from which runs the simple tubular *oesophagus* (*oe.*). The stabber-sac, which lodges the piercing mouth-parts or *stabbers*, is a ventral diverticulum arising anteriorly from the floor of the food canal and runs backwards as far as a diaphragm of tissue which partially separates head and thorax; it is supported posteriorly by a pair of large retractor muscles (*p.s.rt.*). Where the sac leaves the food canal two paired structures, with a certain amount of connective tissue, arise from the walls of the sac as half-tubes (*s.t.*) and run forwards in the buccal funnel as far as the buccal festoons (*b.fs.*). The presence of this *sac tube* was not observed by Harrison who consequently

failed to grasp the true significance of the relationships of the mouth-parts. From the floor of the pumping-pharynx, slightly behind the region where the food canal and sac diverge and dorsal to the sac tube, the paired pharyngeal half-tubes (*ph.t.*) have their origin. These evidently compose Harrison's "buccal tube." These half-tubes by their apposition to one another form a suctorial *pumping-pharyngeal tube* (Text-fig. II) through which blood is drawn during feeding. Anteriorly they are embraced by the sac tube. As the ensheathing sac tube and the suctorial pumping-pharyngeal tube both lie in the buccal region it is suggested that the use of Harrison's term "buccal tube" be discontinued.

The two stabbers, when retracted, rest one above the other along the whole length of the sac. They are similar in so far that each resembles a

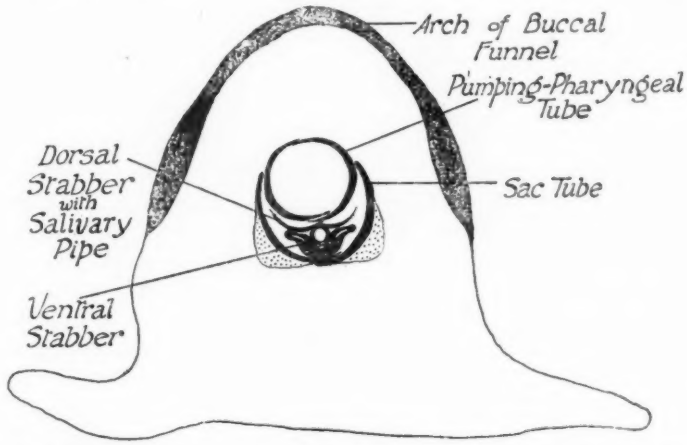

Fig. II. *Pediculus humanus.* Mouth-parts in transverse section at the anterior part of the buccal region. The suctorial tube is situated dorsally to the stabbers and salivary pipe, the complex lying within the hollow of the sac tube. The suctorial and sac tubes are each composed of two elements. Slightly diagrammatic.

two-pronged fork with the rami pointing backwards (Pl. VI, fig. 2). The stabbers are slightly curved and lie completely retracted within the head. In the buccal region they lie beneath the pumping-pharyngeal tube and are embraced by the sac tube. Their posterior rami are connected with muscles and the sides of the sac. Between the stabbers lies the *salivary pipe*, which, according to my sections, appears fused along its anterior length to the upper stabber.

When the louse wishes to feed it would appear as if the parts must function as follows: the stabbers are extruded and thrust for about one-third of their length into the human skin. The buccal arch, pumping-pharynx and pharynx are brought forward, the haustellum is everted and the tips of the pumping-pharyngeal tube and sac tube touch the surface of the epidermis.

Anteriorly the sac tube partially embraces (1) the pumping-pharyngeal tube, and, beneath this, (2) the upper stabber with the salivary pipe and (3) the lower stabber. The pumping-pharynx sucks blood into the pumping-pharyngeal tube. The pharynx may fill and empty three times per second but probably the rate varies greatly.

MORPHOLOGY IN DETAIL.

The structures are described in a resting position but with the haustellum protruded slightly.

Food Canal.

Mouth. The anterior chitin of the head from which the haustellum projects is somewhat thin and loose. The haustellum is hood-like, with a thick base and with a mid-ventral slit, the buccal slit. The chitin of the rim is thin and is continuous with that of the buccal region. Anteriorly the buccal region is made up of two lateral concave dental plates, each bearing eight teeth. The chitinous teeth are placed five laterally and three dorso-laterally on each side. Each resembles a parrot's beak and appears to be based on fibrous tissue. The chitin between the dental plate and the buccal funnel is thin and permits them a certain freedom of movement. When the haustellum is protruded slightly the structures surrounding the mouth are: (1) dorsally and laterally, the buccal funnel (buccal plate of Harrison); (2) ventrally, a floor of chitin continued inwards from the external ventral chitin of the head; (3) the paired buccal festoons which lie just above the floor and are closely approximated in the mid-ventral line.

In the same transverse plane as the base of the haustellum are the buccal festoons. Each *buccal festoon* is a thick bar of chitin curving from the mid-ventral line obliquely outwards, backwards, and upwards to fuse with the lateral chitin at the front of the head. The function and significance of these structures is uncertain.

Buccal funnel. (Pl. VI, fig. 4.) This is a strong chitinous structure, about one-third the length of the head, shaped like a concave capital A with the letter solid above the bar, the point anterior, the concavity facing ventrally and the limbs diverging posteriorly. The anterior arched region forms the roof and sides of the beginning of the food canal and anteriorly it is hinged to the dental plates. The processes of the arch, which are longer than the arched portion, are longitudinally grooved for part of their inner length but the grooving disappears as they taper backwards. The grooved portions form part of the sides of the pumping-pharynx. When the pumping-pharynx contracts posteriorly the processes leave it. The arch is covered by membraneous tissue and is slung anteriorly in the middle line from the roof of the head by strands of similar tissue. The processes serve for the insertion of various muscles.

The muscles associated with the buccal funnel are the protractors and retractors (Pl. VI, fig. 1 and Text-fig. III).

(1) *Protractors*, two pairs: (*a*) dorsal, (*b*) ventral. They serve to bring the buccal funnel forward for feeding. (*a*) Each dorsal muscle (*d.b.pr.*, Text-fig. III) has its origin in the chitin of the anterior dorsal region of the clypeus. Its insertion is along the dorsal surface of the process but it does not quite attain the posterior extremity of the process. (*b*) Each ventral muscle (*v.b.pr.*, Text-fig. III) takes a similar course to the dorsal but ventrally to the process. Its origin is in the chitin of the anterior ventral region of the clypeus and its insertion along the ventral surface of the process. (2) *Retractors*, one pair. Their function is to retract the buccal funnel after feeding. Each muscle (*b.r.*, Text-fig. III) is short, thick, and arises from the lateral chitin of the clypeus. There does not appear to be any one area of insertion as the muscle seems to finish as a fascia sheathing the arch anteriorly.

Pumping-pharynx (*pp.*, Text-fig. III and Pl. VI, fig. 1). This is a bladder of thin chitin, pear-shaped when dilated, of relatively large capacity, lodged within the processes of the buccal funnel and retained in position by several muscles. Its dorsal chitin is collapsed when the insect is not feeding. Its narrow anterior end succeeds the buccal funnel and at its posterior end is a constriction leading to the pharynx. Where the posterior constriction begins, setae are present on the floor and sides. The function of the setae (Pl. VI, fig. 1) is a matter of conjecture[1]. The muscles are dilators and retractors but some seem to act both as dilators and protractors and one acts as dilator and retractor. (1) *Medial dilator-protractors* (*m.d.pr.*, Text-fig. III and Pl. VI, fig. 1)—four pairs. The origins of these muscles are in the roof of the clypeus near the middle line and they follow one another closely. The muscles run almost longitudinally, near the middle line, and their insertions, which succeed each other closely, are in the roof of the pumping pharynx near the middle line.

(2) *Dorso-lateral dilators* (*d.-l. d.*)—three pairs, anterior, median and posterior. Each originates from the dorso-lateral chitin of the head and runs downwards and transversely to its insertion in the roof of the pumping-pharynx. The anterior pair runs down from the posterior dorso-lateral chitin of the clypeus, in the transverse plane, to their insertions on either side of the middle line and close behind the arch of the buccal region. The second pair also runs down in the transverse plane from the dorso-lateral chitin of the head, where the clypeus and epicranium meet, to their pumping-pharyngeal insertion which is a short distance behind that of the anterior pair and external to the dilator-protractor insertions. The third pair runs downwards and anteriorly from the dorso-lateral chitin of the anterior of the epicranium to their insertions at about the widest part of the pumping-pharynx and external to the insertions of the dilator-protractors. (3) *Dorsal retractors* (*d.r.*, Text-fig. III and Pl. VI, fig. 1)—one pair. These are long muscles with

[1] The setae recall the bunches of retrograde hairs protruding into the lumen of the alimentary canal in mosquitoes at a point where the pharyngeal pump joins the oesophagus. See Nuttall and Shipley (*Journ. Hygiene*, 1903, vol. III, p. 198, Pl. VII, figs. 4 and 5), who ascribe a sieve-like function to these hairs.—ED.

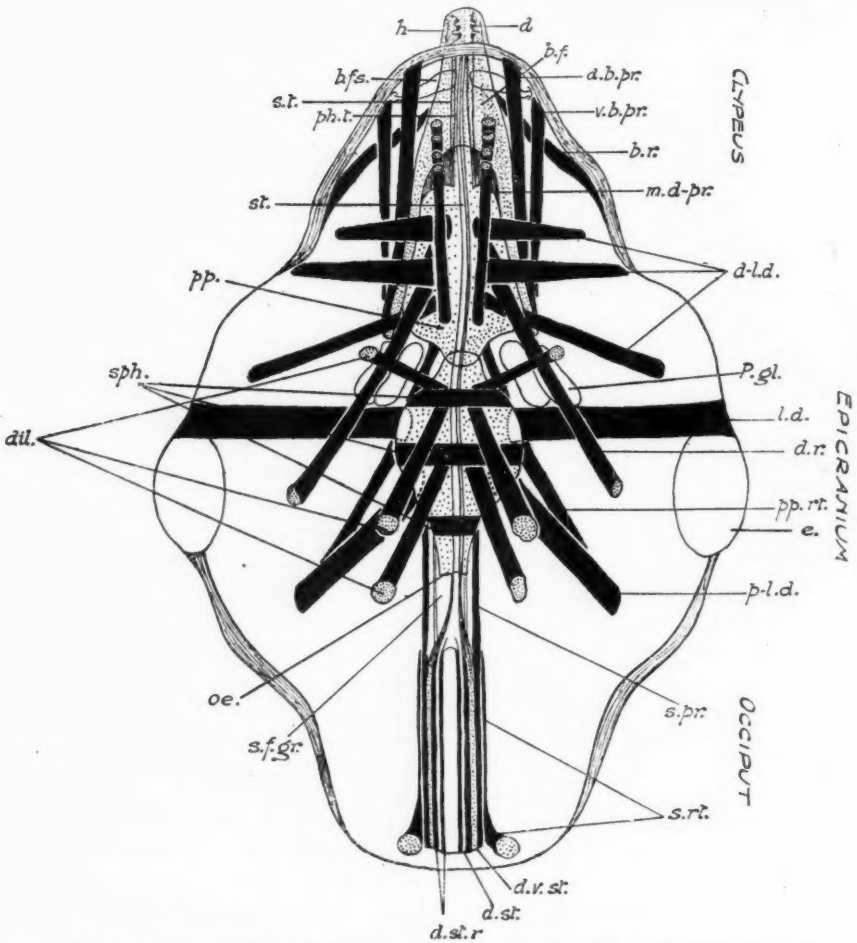

Fig. III. *Pediculus humanus.* Diagrammatic dorsal view of the head. The brain and nerve ganglia are omitted and only the beginning of the oesophagus is represented in order that the underlying stabber-sac and its contents may be revealed; letters taken clockwise.

d. denticles; *b.f.* buccal funnel; *d.b.pr.* dorsal protractor of buccal funnel; *v.b.pr.* ventral protractor of buccal funnel; *b.r.* retractor (?) of buccal funnel; *m.d-pr.* medial dilator-protractor muscles of pumping-pharynx; *d-l.d.* dorso-lateral dilator muscles of pumping-pharynx; *P.gl.* Pawlowsky's glands; *l.d.* lateral dilator muscle of pharynx; *d.r.* dorsal retractor muscle of pumping-pharynx; *pp.rt.* posterior (ventral) retractor of pumping-pharynx; *e.* eye; *p-l.d.* postero-lateral dilator muscle of pharynx; *s.pr.* protractor muscle of sac ("musculus digastricus" of Pawlowsky); *s.rt.* retractor muscle of sac; *d.v.st.* ramus of dorsal element of ventral stabber; *d.st.* ramus of dorsal stabber; *d.st.r.* retractor muscle of dorsal stabber; *s.f.gr.* floor groove of sac; *oe.* oesophagus; *dil.* three dilator muscles of pharynx; *sph.* three sphincter muscles of pharynx; *pp.* pumping-pharynx; *st.* stabbers; *ph.t.* pharyngeal tube; *s.t.* sac tube; *b.fs.* buccal festoon; *h.* haustellum.

origins far back in the epicranium. They are inserted externally at the broadest part of the pumping-pharynx externally to the medial dilators. These appear to be the muscles which Harrison calls "the retractors of the buccal cavity running from the cornua backwards, upwards and outwards." (4) *Ventral retractors*—one pair. They retract the pumping-pharynx after feeding. Each originates in the floor of the head in the same transverse plane as the eyes and is closely associated with the origin of the ventro-lateral dilator of the second pharynx. It runs upward and inward with this dilator for some distance, next runs close under the second pharynx where the girth of the pharynx is greatest, and from thence to its insertion at the posterior portion of the pumping-pharynx where its constriction begins. This retractor is not mentioned by Harrison.

Pumping-pharyngeal tube (*ph.t.*, Pl. VI, fig. 1, Text-fig. III). The paired elements of the tube run forward from the beginning of the floor of the pumping-pharynx nearly to the teeth. Each is a simple half-tube (Pl. VI, fig. 5), crescentic in cross-section, the rims being sharp edged whereby the half-tubes are capable of overlapping one another (Text-fig. II) and anteriorly, perhaps, may be applied to the arch of the buccal funnel. How the two elements are brought together to form a tube I do not know. Harrison suggests that it is done by a "pair of elastic folds, which lie at the base of the tube, and which pass obliquely upwards and backwards."

Pharynx (*ph.*, Pl. VI, fig. 1). This dilatation of the food canal is curiously shaped and musculatured. Distended with blood, it is bulbous, but at rest its floor, walls and roof have each a broad deep furrow. In cross-section the lumen has the shape of a four-rayed star-fish with the two dorsal arms larger than the ventral. The wall is composed of three layers (1) the inner lining of thin chitin, (2) the middle epithelial and chitinogenous layer and (3) the outer muscular layer. The latter appears to include an inner layer of longitudinal fibres, and, in certain areas, special, large, outer, circular sphincter muscles.

The muscles consist of sphincters and dilators disposed as follows; dorsally and in succession backwards—the anterior dorsal dilator, the anterior sphincter, the medial dorsal dilator, the medial sphincter, the post-dorsal dilator, the post-sphincter; laterally and in succession backwards—the lateral dilator between the anterior and medial sphincter, the post-lateral dilator immediately after the medial sphincter and between it and the posterior sphincter.

1. *Sphincters*—three (*sph.*, Pl. VI, fig. 1 and Text-fig. III). (*a*) The anterior sphincter is broad and encircles the pharynx just behind the insertion of the anterior dilator. (*b*) The medial sphincter encircles the pharynx about the beginning of its posterior third. (*c*) The posterior sphincter, the smallest, encircles the pharynx where it tapers to the oesophagus.

2. *Dilators*—five pairs (*a–e*) as follows (*dil.*, Pl. VI, fig. 1 and Text-fig. III). (*a*) Antero-dorsal. Each has its origin in the anterior part of the epicranium

and runs obliquely downward and backward to its insertion in the roof of the pharynx nearly in the middle line and partly under and in front of the anterior sphincter. (*b*) Medial dorsal. Each muscle arises from the roof of the epicranium posteriorly and runs downward to its insertion in the roof of the pharynx immediately behind the anterior sphincter. (*c*) Post-dorsal. These run almost parallel to the medial dilators. Their origins are close behind those of the medials but their insertions are under the medial sphincter. The function of these three pairs is to dilate the pharynx by raising the sagging roof. The anteriors probably assist in protracting the pharynx during feeding while the other two are retractile. (*d*) Lateral (*l.d.*, Text-fig. III). Each is a very thick muscle which originates from in front of and above the eyes, and is inserted widely and strongly in the upper half of the wall of the pharynx at its middle. (*e*) Post-lateral. Each is a thick muscle which originates from the floor of the head posterior to the eyes and runs forward, upward and inward to the insertion in the lower half of the wall of the pharynx between the medial and posterior sphincters. The function of both pairs is to bulge the walls outward, and the post-lateral probably retract the pharynx after feeding.

This account is at variance with Harrison's in which a third dilator and sphincter are not mentioned.

Suction seems therefore to take place as follows: the haustellum is placed in close contact with the host's skin and, with the assistance of the sac tube, air is prevented from entering the food canal. The contraction of the dilators of the pumping-pharynx causes a negative pressure in the latter and the blood is drawn in from the wound. The anterior sphincter controls the entrance to the pharynx, the medial regulates the supply, while the posterior controls the egress. The anterior dilators of the pharynx contract and raise the anterior portion of the sagging roof while the anterior sphincter is relaxed. Blood thus enters the anterior part of the pharynx. The anterior dilators then relax and the anterior sphincter contracts and blood is retained in the centre of the pharynx as the medial, dorsal and lateral dilators contract and increase the volume of that organ. The medial sphincters next relax and the post-dorsal and post-lateral dilators contract and cause blood to enter the posterior part of the pharynx; this of course taking place at the same time as the medial and lateral dilators relax and the medial sphincters contract. The last effort is the relaxing of the posterior sphincter to permit blood to enter the oesophagus, this happening when the medial sphincter contracts and the post-dorsals and post-laterals relax. The whole process recalls that of peristalsis. I have not observed the occurrence of re-gurgitation.

Oesophagus. The pharynx opens into the gullet which is a thin-walled tube, curving upwards in the middle line and running into the thorax where it opens into the stomach.

Stabber-Sac. It is convenient to consider first the relationships of the various structures of the region where food canal and stabber-sac diverge.

The roof of the sac leaves the floor of the food canal beneath the posterior end of the buccal arch anteriorly to the pumping-pharynx. The lumen, seen in cross-section (Text-fig. IV, 6, 7 and 8), shows three regions, (1) the wide, upper pumping-pharyngeal region, (2) the lower, narrower sac region and (3) the still narrower connecting region. The boundaries of the pumping-pharyngeal region are a roof made of the arch of the buccal funnel, a floor of chitin incomplete in the middle line, and the paired elements of the pumping-pharyngeal tube. In cross-section the latter look like round brackets facing one another ( ). The boundaries of the sac are two walls largely made up of the elements of the sac tubes and a thin floor of chitin. The elements of the sac tube in cross-section resemble query marks and face one another ʕ ʔ. Their dorsal overhanging parts act as guides to the upper stabber. The connecting space has thin chitinous walls. The appearance of these structures at various points along their length may be followed by comparing the series of sections illustrated in Fig. IV, 8–4 with Pl. VI, fig. 1.

The tissues and structures which run forward into the buccal region are (*a*) the connective tissue outside the floor of the funnel, (*b*) the pumping-pharyngeal tube, (*c*) the sac tube, and (*d*) the connective and muscular (?) tissues of the wall joining these tubes. Anteriorly to the elements of the sac tube remnants of muscular (?) tissue persist.

Even with the oil-immersion lens it is difficult to recognise the exact nature of the tissue of the sac. For about the first third of its length it appears to consist of extremely thin chitin surrounded by a fine layer of chitinogenous cells, but posteriorly, except in a certain special region of the floor, only a thin blue line is shown under the high power. For the first two-thirds of its length the cavity of the sac is spacious and contains only the stabbers. Posteriorly the sac is almost completely filled owing to the increase in size of the stabbers and the presence of their muscles which have a common origin at the blind end of the sac. This blind end is supported by a pair of retractor muscles whose relationships are discussed later. One part of the floor, roughly speaking the middle half, is specialised as a chitinous groove, the *sac floor groove* (Pl. VI, fig. 2), the edges of which are thickened and flanged. In cross-section (Text-figs. IV, V, 11, 12, 14) it is shaped like a wide capital U with the tips bent outward and thickened. The anterior third is separated from the posterior two-thirds by a suture (Harrison). In the posterior quarter of the sac the groove disappears. An additional pair of grooves is also present in the floor of the sac (Text-fig. IV, 9, 10 and 11). They are laterally placed and reach from a short distance behind the anterior portion of the wide sac groove to a short distance in front. These lateral floor grooves seem associated with Pawlowsky's glands which open into the sac. The other structures pertaining to the sac are Pawlowsky's glands, sac muscles, and the stabbers with their muscles. As the musculature is closely bound up with that of the stabbers it will be described after the stabbers. The salivary glands (*vide infra*) open into the sac posteriorly.

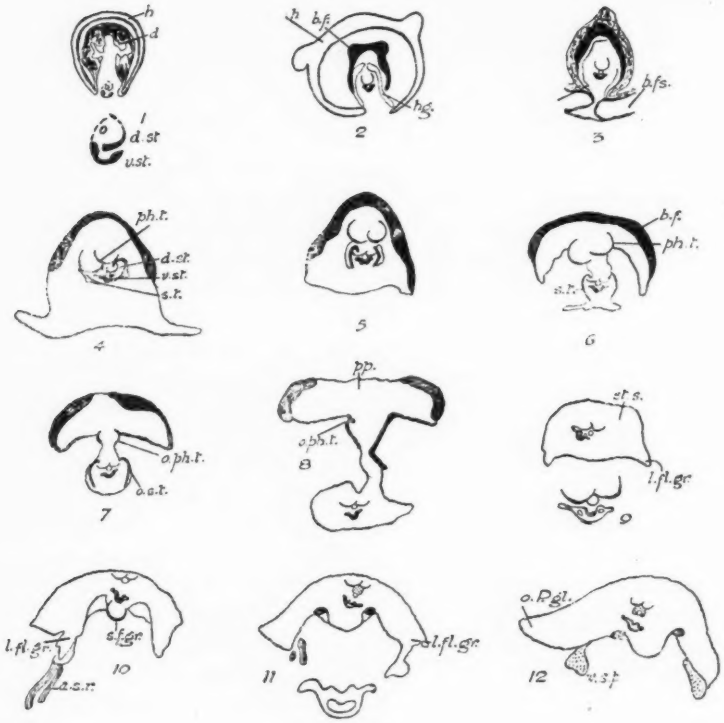

Fig. IV.

Figs. IV, V. *Pediculus humanus.* Series of transverse sections of mouth-parts. Drawings, slightly schematic, from slightly oblique sections; some sections, particularly the posterior ones, show some shrinkage and displacement; the mouth-parts are shown in black; sections 9 to 25 refer to the stabber-sac.

a.s.r. anterior retractor muscle of stabber-sac; *a.v.st.r.* retractor muscle associated with posterior retractor of the stabber-sac and the ramus of the dorsal element of the ventral stabber. *b.f.* buccal funnel; *b.fs.* buccal festoons; *d.* denticles; *d.st.* dorsal stabber; *d.s.pr.* dorsal portion of the protractor muscle of the sac; *d.r.v.st.* ramus of dorsal element of ventral stabber; *d.st.r.* retractor muscle of dorsal stabber; *f.t.* fibrous tissue; *h.* haustellum; *h.g.* hinge of dental plate; *l.fl.gr.* lateral floor groove of stabber-sac; *o.P.gl.* region of orifice of Pawlowsky's glands; *o.ph.t.* origin of pharyngeal tube; *o.s.t.* origin of sac tube; *ph.t.* pharyngeal tube; *pp.* pumping-pharynx; *p.s.r.* posterior retractor muscle of stabber-sac; *p.v.st.r.* retractor muscle associated with the retractor muscle of the dorsal stabber and the posterior of the ramus of the lower element of the ventral stabber. *s.pr.* protractor muscle of the stabber-sac; *v.st.r.* retractor muscle of the ramus of the dorsal element of the ventral stabber; *v.r.v.st.* ramus of ventral element of ventral stabber.

EXPLANATION OF TEXT-FIGURES.

FIGS. IV—V.

Section 1. Dental region; stabbers shown also on larger scale; dorsal stabber broken. *d.st.* dorsal stabber; *v.st.* ventral stabber; *h.* haustellum; *d.* denticles.

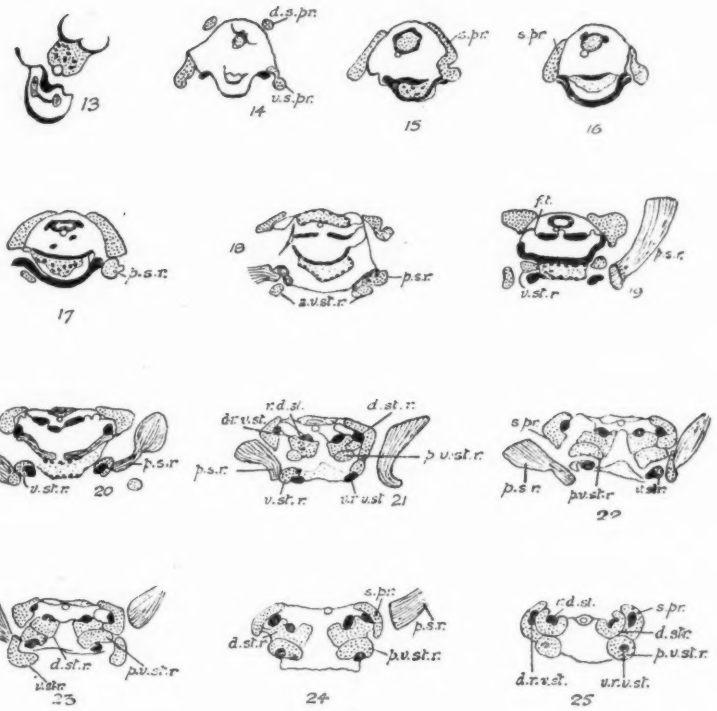

Fig. V.

Section 2. Anterior part of buccal funnel (*b.f.*) and base of haustellum; dorsal stabber forming a single groove; *h.g.* chitinous hinge of dental plate to buccal funnel.

„ 3. Anterior part of buccal funnel and festoons (*b.fs.*).

„ 4. Anterior part of all the mouth-parts in the lumen of buccal region; note how the elements of the pharyngeal tube (*ph.t.*) are dorsally placed and how the elements of the sac tube (*s.t.*) embrace the stabbers; shreds of muscular (?) or connective (?) tissue still adhere to the elements of the sac tube.

„ 5. Showing how the elements of the sac tube act as guides to the stabbers.

„ 6–8. Region of divergence of food canal and stabber-sac; note the origins of the elements of the pharyngeal tube (*o.ph.t.*) and those of the sac tube (*o.s.t.*).

„ 9. Anterior part of stabber-sac; the dorsal stabber is slightly distorted; the drawing below shows how one element overlaps the other and how the fusion of the dorsal and ventral elements of the ventral stabber is not perfect.

„ 10. Anterior extremity of sac floor groove (*s.f.gr.*) somewhat distorted; note the lateral floor grooves (*l.fl.gr.*) and the insertion of the small anterior retractor muscles of the sac (*a.s.r.*) and the widening of the sac at the region of the orifice of Pawlowsky's glands.

„ 11. At region of the orifice of Pawlowsky's glands—orifice not shown (*o.P.gl.*); note the flanged edges of the sac floor groove, the tissue of the dorsal stabber separating it from the salivary pipe, the three dorsal grooves and the lumen of the ventral stabber.

„ 12. Similar to sect. 11; note anterior part of ventral portion of protractor muscle of the sac ("musculus digastricus" of Pawlowsky) (*v.s.pr.*); stabbers obliquely cut.

Section 13. Stabbers at region shown in sect. 12: note the darkly stained dots in the tissue of the dorsal stabber and the tissue of the lumen of the ventral stabber.

,, 14. Showing anterior portion of dorsal (*d.s.pr.*) and ventral (*v.s.pr.*) parts of the protractor muscle of the sac

,, 15. Showing merging of the dorsal and ventral parts of the retractor of the sac; the flattening of the dorsal stabber; the darkly stained peripheral portion of the tissue of the dorsal stabber; the region where the ventral element of the ventral stabber rises up from the floor of the sac.

,, 16. Showing the separation of the lateral elements of the dorsal stabber; the flattening of the dorsal element of the ventral stabber and its separation from its ventral fellow except for a thin connecting membrane.

,, 17. Showing lumen in the tissue of the dorsal stabber; the floor of the sac formed by the ventral element of the ventral stabber only; the insertion of the posterior retractor muscle of the sac (*p.s.r.*); the broadening of the dorsal element of the ventral stabber. The black dots in the lumen are displaced fragments of the dorsal stabber.

,, 18. Parts slightly displaced. Showing dorso-lateral position of the protractor muscle of the sac; the tissue of the dorsal stabber and the salivary pipe *above* the dorsal stabber; the flat, broadening elements of the dorsal stabber; the grooved portion of the dorsal element of the ventral stabber; the rami of the ventral element of the ventral stabber; the two insertions of the retractor muscles of sac (*p.s.r.*), the main muscle and (*a.v.st.r.*) a smaller muscle associated with it.

,, 19. Similar to sect. 18; showing insertion of retractor muscle of the dorsal element of the ventral stabber (*v.st.r.*); fibrous connection of dorsal stabber to sac (*f.t.*) and ventral stabber.

,, 20. Similar to sects. 18 and 19; showing beginning of bifurcation of the dorsal element of the ventral stabber; retractor muscle of dorsal element of ventral stabber (*v.st.r.*) now running along the ramus of the ventral element.

,, 21–23. Showing insertion of the retractor muscle of the dorsal stabber (*d.st.r.*) and the associated muscle (*p.v.st.r.*) which ultimately runs along the posterior part of ventral element of the ventral stabber.

,, 24. Note the disappearance of the retractor muscle of the ventral element of the ventral stabber (*v.st.r.*); the presence of the muscle (*p.v.st.r.*) associated with the retractor of the dorsal stabber. The tissue around the salivary pipe was lost in preparing the section.

,, 25. Similar to sect. 24. The salivary pipe appears to be thickening. The muscles are very close together.

Pawlowsky's Glands (*P.gl.*, Pl. VI, fig. 1 and Text-figs. III, VI). The significance of these glands is not understood. The large orifice of each gland opens into the sac slightly more than half way along its length. The gland secretion appears to be poured into the lateral floor grooves. Each gland lies mainly above and laterally to the sac and resembles a tube looped twice on itself (Text-fig. VI, A). The walls of the tube are thick and consist of one layer of large glandular cells (Text-fig. VI, B). Three regions may be distinguished: (1) the outer and upper third, (2) the medial and (3) the lower and terminal third. The lumen of the first third runs forward, downward, and inward. It loops ventrally into the medial third whose cavity runs backward and downward. Next is another sharp ventral loop leading to the cavity of the terminal third which opens into the sac by a wide orifice and pours the secretion into the lateral floor grooves. I infer that the function of the glands is to provide a lubricant for the stabbers.

Stabbers (Pl. VI, figs. 4 and 5). The stabbers are similar in certain respects. Each resembles a long two-pronged fork, the rami being situated posteriorly. The rami are about one-fifth the total length of the stabbers and their extremities are tendinous. The anterior ends of the stabbers are thin, flexible, and serve for piercing. When retracted the distal portions are slightly curved longitudinally and reach from the blind end of the sac nearly to the teeth and lie between the pumping-pharyngeal tube and the sac tube. The stabbers are equal in length, being slightly longer than the head (0·6 mm.), but through their curving when retracted they are completely accommodated within the head. I cannot follow Harrison in his statement that "the piercing apparatus enters into this tube (the buccal) through the ventral fissure and runs forward within it." Sections and the dorsal aspect of cleared specimens do not show this (Text-figs. III, IV).

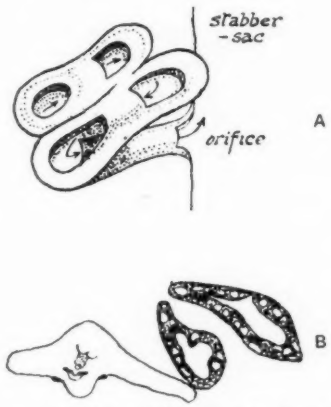

Fig. VI. *Pediculus humanus.* (A) Schematic figure of Pawlowsky's gland as described in the text. (B) Cross section of gland and stabber sac.

The Dorsal Stabber is almost bilaterally symmetrical and formed by the close apposition and the anterior fusion of the two inner edges of two similar elements. The rami run forward from the blind end of the sac and occupy a more median position than the rami of the lower stabber, the span of their bifurcation is the narrowest of the three spans of the stabber bifurcations. The rami broaden, converge and nearly meet in the middle line. Fibrous tissue connects them with one another and with the walls of the sac, thus forming a single flat surface anterior to the bifurcation (Text-fig. V, 18–23). From this region the stabber tapers distally. The flat plate is short and a spindle of tissue traverses and divides it into two elements. These are rod-like for a short distance when lateral to the tissue, but, on running forward, they broaden inwardly and become grooved with their gouge-like concavities facing dorsally. These two gouges fuse along the middle line but the inner edge of the right element overlaps that of the left a little, thus giving a slightly

asymmetrical appearance to the stabber. When the stabber is isolated the double gouge shows a blade broader and thinner than the blades of the ventral stabber. Near the piercing end, the two elements become singly concave and bear a three-pointed tip, the centre point large and the flanking ones smaller, the whole recalling the appearance of the needle point of a syringe. I think that the salivary pipe becomes part of this point, as sections and dissections do not show any separation of salivary pipe and stabber.

The above-mentioned spindle of tissue is of interest. It begins as an aggregation of a few cells attached to, and beneath, the roof of the postero-median portion of the sac just above the region of the bifurcation. These cells stain blue and their nuclei almost black. The spindle runs forward above and separates from the dorsal stabber, and certain sections show the presence of a lumen; it then traverses the elements of the dorsal stabber and runs forward whilst attached ventrally to them. The spindle carries attached beneath it a chitinous tube, the salivary pipe, and ceases about the end of the anterior third of the stabber. At its widest region, in cross-sections, it shows a reddish stained core which appears to consist of longitudinal muscle fibres surrounded by a layer of blue-stained cells. Anteriorly the tissue stains pink and contains two groups of blue-stained cells. The function of the tissue is considered later.

Salivary Pipe. As already mentioned the salivary pipe is a chitinous tube of very fine bore running beneath the dorsal stabber tissue. As this tissue diminishes and ceases anteriorly the pipe is brought nearer to, and I think, is fused mid-ventrally to the elements of the upper stabber in the angle between them. A drawing made by Professor Nuttall and a dissection by Mr Lloyd of the posterior end of the pipe show a swelling in this region, and Harrison also pictures it. In my opinion Mr Lloyd's dissection demonstrates that into this bulbous structure open the four salivary ducts. The function of the pipe is to lead the saliva into the wound. Its relationships with other structures are considered later.

Ventral Stabber. According to my dissections and sections this stabber is composed of two similar elements, *dorsal and ventral.* Harrison does not show this. Each element closely resembles the dorsal stabber.

The posterior rami of the *dorsal element* have a wider span than those of the dorsal stabber. They are somewhat bow-shaped, with two curves, and run forwards, externally to those of the upper stabber, and their convergence and fusion take place slightly behind and beneath the upper stabber's broad region. Here the structure has a short, broad, shallow groove facing dorsally. Anteriorly again, the chitin flattens and tapers and the groove disappears. Next, in the piercing portion appear three dorsal longitudinal grooves (only one of which is represented in Pl. VI, fig. 2), one median and two lateral. The median groove persists along the whole length of the stabber and lodges the salivary pipe, but the others become gradually less pronounced and, in the anterior fifth of the stabber, are scarcely recognisable. The edges are flanged. Harrison regards them as crenate but dissections and mounted preparations

do not show this. The tip consists of two minute mandibular dilatations toothed on the outer edges.

The *ventral element* commences as two rami, ventral and internal to those of the dorsal elements. They are straight and have the widest span of all the paired rami. They run forward in the floor of the sac, then converge and grow wider inwards till they fuse as a groove which fits exactly into the sac floor groove. So exact is the fit that for a long time I was inclined to think that the ventral element was a "split away" from a sac groove which, as figured by Harrison, had posterior rami. Further investigation, however, shows that the sac floor groove ends bluntly and that the posterior rami belong to the ventral element of the ventral stabber. The tip is similar to that of the dorsal element, only the mandibular dilatations are larger and outermost.

At first the two elements of the ventral stabber fuse along their margins only but ultimately they fuse along their length. The anterior two-thirds of the ventral stabber is therefore composed of two elements, dorsal and ventral, each element having a separate origin posteriorly. Cross-sections of the stabber anteriorly show that fusion is not complete as two minute cracks are visible in the chitin, each one beneath what is left of the lateral grooves. In dissections, too, the elements may readily be split from one another.

The space between the upper and lower portions of the stabber, before their fusion, is occupied by tissue which commences posteriorly, as connective tissue, similar to the general connective tissue of the head, and is packed between the posterior muscles of the stabber and the floor of the sac. Beneath the region where the dorsal groove begins, it becomes a distinctly characteristic tissue, consisting largely of blue-staining cells with very dark-staining nuclei. Further forward the tissue stains red and appears to be similar to that of the dorsal stabber.

The function of these stabber tissues is probably twofold in (1) bending or turning of the stabbers while in action so as to assist boring into the skin of the host, (2) regenerating, in the larval stages, the stabbers, before ecdysis.

Stabber Muscles (Pl. VI, fig. 1, Text-figs. III–V). These consist of one pair of protractors which operate both the stabbers and the sac, two pairs of retractors of the sac, one pair of retractors of the dorsal stabber and one pair of retractors of the ventral stabber.

(1) Protractors—one pair (*s.pr.*). These are the "musculi digastrici" of Pawlowsky who does not describe their nature and function exactly. Each muscle has two origins on the sac, one dorsal and the other ventral. The dorsal origin is situated slightly to the side and above the orifice of Pawlowsky's gland. The ventral origin is anterior to the dorsal and is situated nearly as far forward as the anterior part of the sac floor groove and inside the lateral floor groove. The two branches run backwards and gradually converge to unite on the side of the sac about half way along its length (Pl. VI, fig. 1, Text-figs. IV, V). The muscle then runs along the wall and gradually rises

till it occupies a dorsal corner position (Text-fig. V, 17–25). At this place the following structures become closely associated—the broad posterior region of the dorsal stabber, the broad region of the upper element of the ventral stabber, the sac wall and the protractor muscle. The muscle pulls upon the sac and stabbers in this region. A thin sheet of fibrous tissue attaches the sides of the stabbers to the sac wall. A short distance behind this, the broad areas cease, the stabbers bifurcate and the rami of the dorsal stabber cross and run internally to those of the upper element of the ventral stabber. The muscle, therefore, is continued backwards along the upper ramus of the ventral stabber only. By their contraction the protractors pull upon the posterior lengths of the sac and stabbers. Their effect upon the sac and stabbers is considered later.

(2) Retractors (Plate VI, fig. 1, Text-figs. III, IV). The following classification of these muscles is for convenience of description, as some muscles and mouth-parts are so intimately associated that it is difficult to give short descriptive names. The muscles all have a common origin at the blind end of the sac. In my opinion certain muscles appear to be double and each moiety influences a different chitinous structure in order to secure co-ordination of movement of the different parts of the mechanism.

A. Retractors of sac—two pairs, (*a*) anterior and (*b*) posterior. (*a*) Each anterior retractor is a short, double muscle originating in the lateral floor of the head posteriorly beneath the lateral floor grooves and running forward, upward and inward to the sac. The insertions are placed one on either side of the lateral floor grooves and about half way along their length. Their function is to retract the anterior portion of the sac when feeding is finished. (*b*) Each posterior retractor arises dorso-laterally in the head, near its junction with the thorax, it runs ventrally and slightly inwards and there appear to be two insertions, one beneath the posterior end of the sac floor groove and the other close beside it on the ramus but ventrally and slightly posteriorly. The dorsal main muscle runs along the sac wall and the ramus of the lower element of the ventral stabber. The branch muscle runs ventrally to the main muscle along the ramus of the lower element of the ventral stabber to a ventral insertion at the distal end of that ramus. When the sac is drawn forward in feeding the retractors are stretched, but when feeding is finished their contraction helps to retract the sac and ventral stabber.

B. Retractors of dorsal stabber—one pair. These muscles appear to have a double origin at the blind end of the sac. One branch, the dorsal, runs along beneath the ramus of the dorsal stabber and on the inner side of the ramus of the dorsal element of the ventral stabber. Its insertion is beneath the distal end of the ramus of the dorsal stabber. Merging with it is a ventral branch which is adherent to the posterior portion of the ramus of the ventral element of the ventral stabber. What influence this branch has on the ventral element of the ventral stabber I cannot say.

C. Retractors of ventral stabber—one pair. Each retractor of the rami

runs along the side of the posterior part of the sac, along and above the ramus of the ventral element of the ventral stabber, to its insertion under the distal end of the dorsal element.

The **Salivary Glands** are situated laterally at about the middle of the thorax and consist of two pairs of glands, one pair bifurcated and tubular and the other reniform.

The bifurcated tubular glands lie above the anterior stomach pockets and are deeply embedded in fat-cells which closely invest the oesophagus and the anterior of the stomach and its pockets. They are about 0·1 mm. in length, and possess a long duct which runs forward to the stabber sac.

The reniform glands are shorter, lie nearer the median line inner and slightly posterior to the tubular glands between the stomach pockets and the oesophagus. From the hilum of each gland there arises a duct which runs forward and parallel with the duct of the bifurcated gland.

The terminations of the salivary ducts in the region of the posterior end of the stabber-sac are of interest. Martin (1913, p. 46) figures the four ducts entering the stabber-sac posteriorly, the figure having been drawn from a dissection made by Bacot. Lloyd's preparation also demonstrates this but shows, to my judgment, that the ducts enter the posterior dilatation of the salivary pipe.

MANNER OF FEEDING.

When the louse desires to feed, certain parts of the mouth apparatus have to be brought externally into action from their concealed positions in the head, and what probably happens is as follows. The contraction of the buccal and pumping-pharyngeal protractors causes the food canal and stabber-sac to be brought forward. The sac must be affected because its chitin is continuous with that of the buccal region: (1) the buccal funnel and the attached pumping-pharynx are brought forward; (2) the dental region becomes everted so that the hood-like haustellum and the teeth are placed externally; (3) the post-dental region comes to touch the skin of the host; (4) the elements of the pumping-pharyngeal tube, by reason of their union with the pumping-pharynx, are brought forward and touch the skin also; (5) the elements of the sac tube are also brought far forward to this point. The contraction of the sac protractors brings the stabbers into play and also influences the sac. Though I have no preparations proving it what probably happens to the sac is that (1) the posterior part of the thin roof becomes telescoped; (2) the sac floor groove reaches a forward position so that the anterior extremity lies beneath the posterior extremities of the elements of the sac tube; (3) the lateral floor grooves are moved forward also to lie beneath these elements; (4) the thin sac floor anterior to the grooves also becomes telescoped.

Because of the structure of the tips of the stabbers they produce a lacerated wound. Whether there is any boring motion or a repetition of stabbing by the prickers in the wound I cannot say. From their small size

and position the buccal teeth merely appear to serve as anchors, but as Harrison suggests they may help to stretch the skin of the host. The saliva, which is probably injected at the deepest part of the wound, may serve as an irritant to stimulate haemorrhage from the capillaries or as an agent for preventing the coagulation of blood in the interior of the insect[1].

From the nature of their cells and the course of the secretion along the floor grooves to the sac tube, it would appear, on the other hand, that Pawlowsky's glands produce a lubricant for the stabbers.

By the pumping action of the pharynx, already explained, the blood is sucked up the afferent channel and passed into the alimentary canal. Anteriorly this afferent channel is possibly made up of the half-tubes of the pumping-pharyngeal tube and the buccal arch and, posteriorly, of the pumping-pharyngeal tube alone.

When feeding is finished the various organs are withdrawn by their retractor muscles.

CONCLUSIONS.

It will be of interest to see what interpretations and homologies will be based on the foregoing description of the mouth-parts. It is hoped that the description will be of use to investigators who wish to ascertain through which channel the organisms of disease may find passage on the way from the insect to man.

REFERENCES

HARRISON (1916), MARTIN (1913) and PEACOCK (1916), see Bibliography in *Parasitology*, x. pp. 1–421.

EXPLANATION OF PLATE VI.

PEDICULUS HUMANUS. STRUCTURE OF THE HEAD.

Fig. 1. The diagram represents a dissection of the right half of the head. Only the right half of the following structures in the mid-dorsal plane are shown—the buccal funnel, the food canal, the stabber-sac with the two stabbers, and the nerve ganglia. For clearness the structures of the stabber-sac, particularly those of the posterior end, are slightly displaced and distorted.

Food canal (beginning anteriorly on first line). *h.* haustellum; *d.* denticles; *d.pl.* dental plate hinged to *b.f.* buccal funnel; *b.fs.* buccal festoons; *ph.t.* pharyngeal tube; *f.t.* fibrous tissue supporting buccal funnel; *b.pr.* dorsal protractor muscle of the buccal funnel; *m.d-pr.* four medial dilator-protractor muscles of the pumping-pharynx; *d-l.d.* three dorso-lateral dilators of the pumping-pharynx; *pp.* pumping-pharynx with setae; *d.r.* dorsal retractor muscle of the pumping-pharynx; *ph.* pharynx; *sph.* three sphincter muscles of the pharynx; *dil.* three dilator muscles of the pumping-pharynx. On the second line—*pp.rt.* posterior retractor of the pumping-pharynx; *p-l.d.* postero-lateral dilator of the pharynx; *b.fs.* buccal festoons.

Stabber-sac (beginning anteriorly on second line). *s.t.* sac tube; *d.s.t.* dorsal stabber running beneath post-dorsal arch of sac tube; *st.c.* stabber-sac; *d.st.t.* tissue of dorsal stabber

[1] See in this connection Nuttall's description of the feeding habits, *Parasitology*, x. 171, and the demonstration by him (*Ibid.* p. 74) that the salivary glands contain anti-coagulins.

with salivary pipe beneath; *l.f.gr.* lateral floor groove of sac; *a.s.rt.* anterior retractor muscles of sac; *s.pr.* ventral origin of the protractor muscle of the sac—on outer wall of sac and shown by dotted lines ("musculus digastricus" of Pawlowsky); *s.f.gr.* sac floor groove; *P.gl.* Pawlowsky's glands with *s.pr.* the dorsal origin of the protractor muscle of the sac beneath—course shown by dotted lines; *v.st.* (*f.*) region of fusion of dorsal and ventral elements of the ventral stabber; *v.st.t.* ventral stabber tissue; *i.p.s.rt.* region of insertion of posterior retractor of sac; *d.st.* dorsal stabber—flattened portion; *d.v.st.* dorsal element of ventral stabber—grooved; *a.v.st.r.* muscle associated with the large posterior retractor muscle of sac and also with ramus of lower element of ventral stabber; *v.st.r.* retractor muscle of dorsal element of ventral stabber; *v.v.st.* ramus of ventral element of ventral stabber; *p.v.st.r.* muscle associated with retractor muscle of dorsal stabber also with posterior extremity of ramus of ventral element of ventral stabber; *d.v.st.r.* ramus of dorsal element of ventral stabber; *d.st.rt.* retractor muscle of dorsal stabber; *s.pr.* posterior portion of retractor muscle of sac (on the outside of sac); *sal.p.* posterior part of salivary pipe; *d.st.r.* ramus of dorsal stabber; *p.s.rt.* posterior retractor muscle of sac—note its anterior insertion *i.p.s.rt.*

General. *br.* brain; nervous tissue all white; *f.b.* fat body; *ch.l.* chitinogenous layer *b.m.* basement membrane; the following muscles cannot be shown on the diagram—the retractor and ventral protractor of the buccal funnel; lateral dilator of pharynx.

Figs. 2–6. The isolated mouth-parts as described by the lettering accompanying the figures. Figs. 2, 5, 6, drawn slightly in perspective. In Fig. 2 the rami of the stabbers and salivary pipe are slightly displaced.

CLAVELLA SCIATHERICA, N. SP., A PARASITIC COPEPOD OF *GADUS MORRHUA*.

By W. HAROLD LEIGH-SHARPE, B.Sc. (Lond.).

(With 8 Text-figures.)

Habitat and Record.

The animal under consideration belonging to the family Lernaeopodidae was taken on a codling (*Gadus morrhua*), 13 ins. in length, caught at Plymouth several years ago, and preserved in formalin since that date. Although about twenty-six valid species of *Clavella* have been either recorded or described in detail, the subject of the present paper differs from them all in combining several of the more prominent characters of other species, notably the extreme length of the backwardly-arched cephalothorax taken together with the reduction of the "arms" (second maxillae) and their expansion into a disc so that the "bulla" or button appears to be almost directly affixed to the body. For this species I accordingly propose the name *Clavella sciatherica*.

One female specimen was taken from the first left gill slit, a second from the second right gill slit, a third (the longest) from the floor of the pharynx, while from the crescentic cushion upon which are borne the vomerine teeth two immature females without ovisacs, with two males adhering to each as indicated in Fig. 5, were obtained[1].

The Females.

Body. The outline of the animal is best seen from Figs. 1 and 2. The length of the specimens differs considerably, but the proportions are constant. The dimensions of the medium-sized specimen are: *Cephalothorax* 5 mm., *Trunk* 2 mm., *Ovisacs* nearly 4 mm.

The general colour and appearance is that usually exhibited in this family. The trunk is a clear white while the ovisacs are yellow.

The *Cephalothorax* is greatly elongated, being more than twice as long as the trunk. It is deflected backwards in a characteristically arched manner, but not so as to touch the dorsal surface of the trunk. It is unprovided with a dorsal carapace and forms a "neck" which is dilated at the base where it joins the trunk from which it is distinctly separated.

[1] Since recording the above, Michael G. L. Perkins, who followed my work, has presented me with upwards of a dozen specimens of *Clavella*, all of them of this new species though considerably larger in size, taken from various codling caught at Lowestoft in August 1918. The animals were located either on the roof of the buccal cavity, or on the floor of the pharynx, at the bases of the gill-arches, *never* on the gill-filaments.

The *Trunk* is almost spherical, resembling the seed of a pea. In front view, however, it is noticeably longer than it is broad. It is flattened dorsi-ventrally, but bears neither abdominal appendages nor anal laminae. The abdomen is represented by a pronounced genital process, ventral to the anus, bearing a median vulva which connects directly with the spermothecal sac.

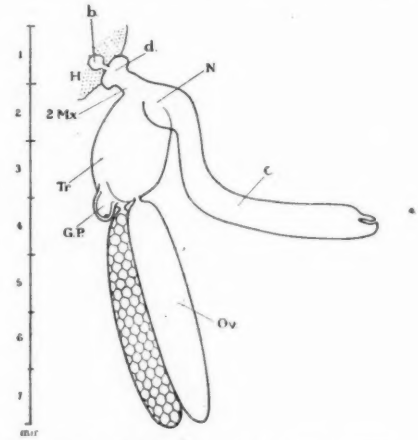

Fig. 1. *Clavella sciatherica*, ♀, in lateral aspect. *C.* cephalothorax; *N.* swelling at the base of the cephalothorax forming a neck; *Tr.* trunk; 2*Mx.* 2nd maxillae; *d.* disc; *b.* bulla; *H.* host; *G.P.* genital process; *Ov.* ovisacs.

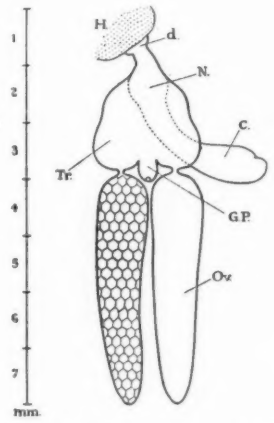

Fig. 2. *Clavella sciatherica*, ♀, in ventral aspect. *C.* cephalothorax; *N.* neck; *d.* disc; *H* host; *Tr.* trunk; *G.P* genital process; *Ov.* ovisacs.

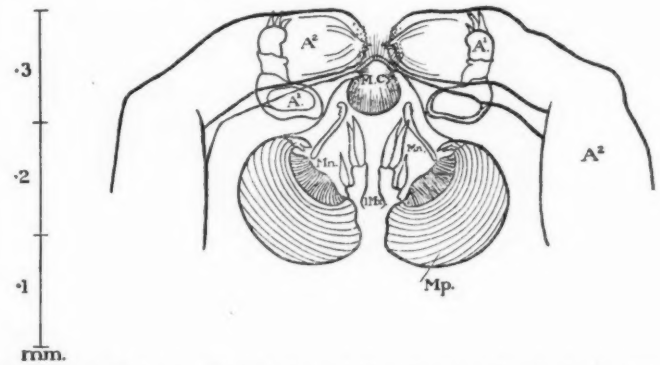

Fig. 3. *Clavella sciatherica*, ♀. The anterior end of an immature female showing some of the appendages *in situ*. *M.C.* mouth cone; A^1. antennule; A^2. antenna; 1*Mx.* 1st maxilla; *Mp.* maxillipede; *Mn.* mandible.

The **Appendages,** all paired (in both sexes) are: the first Antennae (Antennules), the Second Antennae, Mandibles, First Maxillae, Second Maxillae, and one pair of Maxillipedes (Fig. 3).

The 1*st Antennae*, or *antennules*, are small and three-jointed. They are so short that they do not project beyond the antennae. The basal joint is the largest and has a slight turn upon itself. The terminal joint is slightly the larger of the other two, and is tipped with two large spines, with a smaller spine on the inner border.

The 2*nd Antennae* are uniramose, without an exopodite or palp such as is present in *Lernaeopoda*. They are turned inwards towards each other across the frontal margin of the head. They often meet and overlap in the middle line, but do not appear to be deflected in such a way as to do so in this species. They are bluntly rounded at the apex which is covered by a large number of denticulations and are tipped with two or sometimes three very small spines.

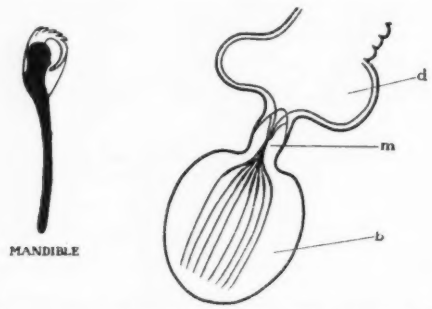

Fig. 4. *Clavella sciatherica*, ♀. The mandible, and the distal end of the 2nd maxillae. *d.* disc; *b.* bulla; *m.* manubrium.

The *Mandibles* appear to be very rudimentary, crotchet-like, bearing five or six teeth on their *outer* margin. In Fig. 4 the part blackened in is strongly chitinised; and the remainder may possibly become so in the course of time, as this is drawn from the immature female. The mandibles are entirely dissimilar from the usual type found in this family.

The 1*st Maxillae* are small, and bipartite with an exopodite or palp such as is present in *Lernaeopoda*.

The 2*nd Maxillae* are entirely fused, very short, not in the same straight line with the cephalothorax, and expanded at the apex into a flat (not cupuliform) *disc* which touches, but does not penetrate, the tissues of the host. From the disc arises a horn-coloured almost spherical *bulla* completely imbedded among the host's cells. The sphere, which exhibits characteristic markings, is joined to the disc by a short *manubrium*, which, like the bulla, but not the disc, is formed of chitin (Fig. 4). During development the 1st maxillae have migrated considerably posteriorly, so that they come to lie at the base of the neck a long distance behind the maxillipedes.

The *Maxillipedes* are clawed appendages whose bases diverge from one another. Each consists of a basal joint which is so stout as to be reniform, well supplied with powerful muscles which move the terminal clawed joint.

which is small, bearing a yet smaller accessory claw, and flex it down against the basal joint. These appendages appear to have undergone considerable outward rotation from the position normally found in this family. Being but feebly clawed, they seem to have lost their functions both of attachment and of feeding.

The **Mouth parts** (Fig. 3). The *mouth*, which is tubular, is situated at the summit of a cone, short in the female, but more than usually long in the male (Fig. 7). It forms a suctorial proboscis, and is composed of an upper and a lower lip, fringed with numerous ordinary setae. The lips are joined in the same manner in both sexes, and present no differences from the condition that obtains in *Lernaeopoda*.

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The **immature females,** each individual bearing two pigmy males (Fig. 5), are of special interest, though such a condition has been previously

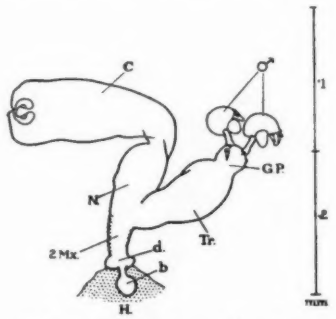

Fig. 5. *Clavella sciatherica*. An immature female carrying two pigmy males (♂). *C.* cephalothorax; *N.* neck; *2Mx.* second maxilla; *b.* bulla; *d.* disc; *Tr.* trunk; *G.P.* genital process (abdomen); *H.* host.

recorded, and superficially figured in *C. uncinata*, and is said to be not uncommon in that species. The *cephalothorax* is, at this early stage, strikingly elongated, but the slightly expanded head (mentioned as a characteristic of other species) may be due to compression on mounting it as a slide for microscopical examination. The *trunk* is more vase-like than in the adult, and the coelomic cavities that will eventually become the ovaries are already recognizable within it. At the posterior end a spherical abdomen is present which has not dwindled down to a mere genital process. The abdomen is separated from the trunk by a deep constriction. At the posterior pole of the abdomen is situated the anus, the posterior end of the alimentary canal being difficult of recognition as it is void of faecal matter. Lateral in position, some little distance from the anus on either side, are the projecting tumid lips of the apertures from which the eggstrings or ovisacs will presently emerge. They possess a crenated margin, which may possibly be an artefact due to shrinkage in the Farrant's medium.

The details of the reproductive system appear to be markedly different from those which obtain in *Lernaeopoda*. Instead of two separate *vulvae*, or apertures to the spermothecal ducts, there is but one prominent median opening surrounded by a funnel-like collar, which appears to represent the two vulvae fused. This leads into an enormously dilated *spermotheca*, the imperfect division of which down its median line seems to indicate its dual origin from two separate cavities. On either side of this division a *spermatophore* has been lodged presumably by the attached males. Into the upper portion of the spermotheca enter the oviducts, while posterior to their entrance they quit this chamber, and, approaching close to the abdomen wall, terminate on either side of the anus at the point where the ovisacs will subsequently be suspended. The proximal (Fig. 6 *Od.*) and distal (*Od*^{1}.)

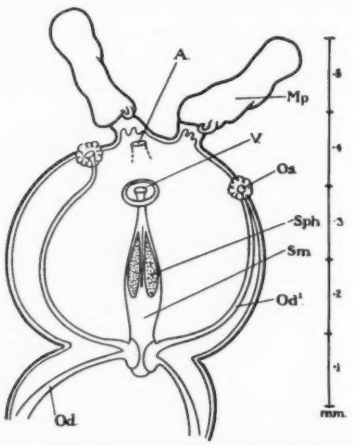

Fig. 6. *Clavella sciatherica*, ♀. The posterior end of the immature female showing part of the reproductive system. *Mp.* the maxillipedes of the males; *V.* vulva; *Od.* proximal portion of the oviduct; *Od*^{1}. distal portion of the oviduct; *A.* anus; *Os.* apertures from which the ovisacs will emerge; *Sm.* spermotheca; *Sph.* spermatophore.

elements of the oviduct are arranged at such an angle that it is conceivable that the ova of one side pass across the spermotheca for fertilisation and out on the opposite side.

The *males* are each attached, by their maxillipedes, to the posterior region of the abdomen in the vicinity of the vulva, on either side of the anus. By means of an inwardly curved hook, which is minute and falciform, they pinch up the body wall of the abdomen into a little papilla. The vulva could be almost completely surrounded by the maxillipedes, which, when placed in apposition, have the conformation of an imperfectly closed tube suitable for the conveyance of a spermatophore.

The *ovisacs* are long and slender, but do not taper; twice the length of the trunk, and containing eight rows of closely packed ova, 22 to 24 in a row.

The Male (Fig. 7).

Body. The cephalothorax and trunk are fused and folded together ventrally into an unsegmented ovoid without distinction of parts, so that the profile of the dorsum resembles that of *Cypris*, or has the general contour of a skull. The males of the genus *Clavella* are singularly uniform in appearance so that specific differences between them are not marked. The dorsum protrudes more posteriorly than in *C. uncinata*. There is neither dorsal carapace, nor abdominal appendages, so that the males of *Clavella* resemble those of *Brachiella* rather than those of *Lernaeopoda*, and even more particularly in

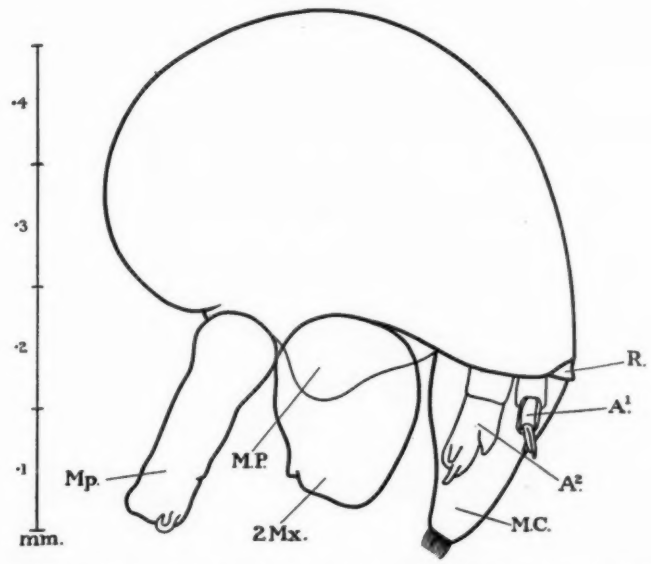

Fig. 7. *Clavella sciatherica*, ♂, in lateral aspect. *R.* rostrum; A^1. antennule; A^2. antenna; *M.C.* mouth cone; 2*Mx.* 2nd maxilla; *M.P.* mediative process; *Mp.* maxillipede.

their appendages. Like *Brachiella* also they are of much smaller size in proportion to the female than in *Lernaeopoda*, being only just visible to the unaided eye. The dorsum is boldly arched, and the height is greater than the length. Between the maxillipedes and 2nd maxillae in the median line a short rounded mediative process like a carina is present, which is homologous with the paired structures similarly situated in *Lernaeopoda*. This process is not located so anteriorly as other authors figure it in other species, *e.g. C. uncinata*; it is, on the contrary, in this species, in a position more in harmony with its above-mentioned homology. All the appendages *in situ* point diagonally and forwards. The *mouth cone* is unusually long and the *rostrum* minute. Greatest length of the body ·35 mm.

The **Appendages,** all paired, are (Fig. 8):

The 1*st Antennae*, or *Antennules*, are two-jointed, the joints being of equal length. The terminal joint is tipped with three long, tapering, flexible, sensitive rami.

The 2*nd Antennae* are uniramose, and three-jointed. There is a smooth prominence where an exopodite is absent. The antenna is rounded at the apex, and tipped with two spines, with a shorter spine on the outer border.

The *Mandibles* appear to be absent, and considering their great degeneration in the female this is not unlikely, though they have been figured in other species. The point cannot be put to the proof without sacrificing the specimens.

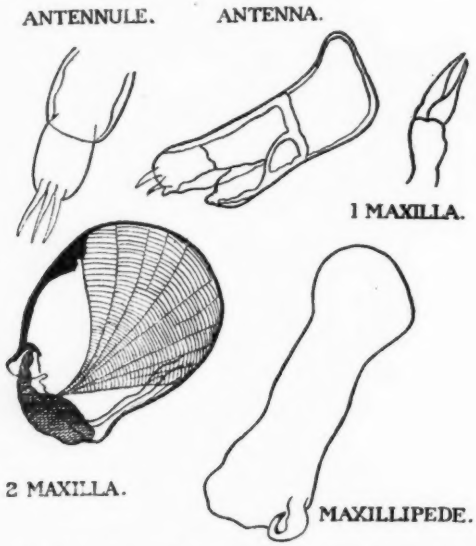

Fig. 8. *Clavella sciatherica*, ♂. Some of the appendages. The antennule, antenna, 1st maxilla, 2nd maxilla, and maxillipede.

The 1*st Maxillae* are bipartite, and do not possess a palp. Each ramus terminates in a delicate point which projects outwards and forwards immediately below the rostrum (omitted for the sake of clearness from Fig. 7).

The 2*nd Maxillae* are close to the mouth tube and almost circular in general outline. They bear a strong resemblance to those of *Lernaeopoda*, and are similarly furnished with a powerful incurved claw at the base which flexes into a protecting sheath.

The *Maxillipedes* are close behind the 2nd maxillae which they do not resemble, as they do in *Lernaeopoda*, but are somewhat like a femur in outline, having a rounded proximal end, and a slightly notched or cleft distal end, which is furnished with a small abruptly curved inturned hook. The maxillipedes are the organs of attachment to the female.

Generic characters.

Female. *Cephalothorax* distinctly separated from the trunk, long, vermiform, slender, arched posteriorly to the body axis, and unprovided with a dorsal carapace. *No* abdominal appendages, or anal laminae; the abdomen reduced to an unpaired genital process ventral to the anus. *1st antennae*, three jointed. *2nd antennae*, uniramose, turned inwards towards each other across the frontal margin of the head, and often meeting and even overlapping in the middle line. *1st maxillae*, bipartite with a palp. *2nd maxillae*, entirely fused, very short (sometimes absent in some spp.). *Maxillipedes*, stout with terminal claw, usually with an accessory claw on the inner margin.

Male. *Cephalothorax* and trunk fused and folded together ventrally into an unsegmented Cyprian ovoid, without distinction of parts. No dorsal carapace or abdominal appendages. *1st antennae* two jointed. *2nd antennae*, uniramose and three jointed. *1st maxillae*, bipartite, without palp. *2nd maxillae* and *maxillipedes* close to mouth tube, and strongly clawed. Between the maxillipedes in the median line, a short rounded mediative process homologous with the paired structures similarly situated in the males of *Lernaeopoda*. All the appendages point diagonally and forwards.

Specific characters.

Female. *Cephalothorax* much longer than the trunk but not deflected backwards so as to touch it. The base of the neck differentiated into a globular swelling, where it is distinctly separated from the trunk. *Trunk* definitely longer than wide. *2nd maxillae* short, completely fused, ending in a disc not quite on the body to which is attached a horn-coloured spherical bulla. The *mandibles* are entirely dissimilar from those figured of other species. *Ovisacs* long, nearly twice as long as the trunk. Genital process present.

There is no species hitherto described which combines all these characters, *C. uncinata* (O. F. Müller), which is also found on Gadoid fishes perhaps approximating most nearly, but that not very closely. The American species *C. irina* Wilson, likewise parasitic on Gadoids, approaches the present species in those points in which it differs most from *C. uncinata*.

The following table gives a comparison between these three species:

	C. sciatherica	*C. uncinata*	*C. irina*
Cephalothorax	*Much* longer than trunk	The same length or but little longer than the trunk	Longer than the trunk
Head	*Not* enlarged	*Not* enlarged	*Not* enlarged
Base of neck	Differentiated	*Not* differentiated	*Not* differentiated
Trunk	Nearly spherical, but a little longer than wide	Wider than long	Much longer than wide
2nd maxillae	Short, with a flat disc as well as a spherical bulla	Short, bulla spherical	Moderately short, bulla small and clavate

	C. sciatherica	*C. uncinata*	*C. irina*
2nd maxillae	*Not* in line with the cephalothorax	In line with the cephalothorax	*Not* in line with the cephalothorax
Genital process	Large	Present, minute	Trilobate
Ovisacs	Long and slender, but do not taper. Twice the length of the trunk	Tapering, from one to two and a half times as long as the trunk	As long as the trunk, tapering
	Ova: about 8 rows of 22–24 in a row	Ova: 10 or 12 rows of 15 to 35 in a row	Ova: 6 rows of 40 in a row

Male. Dorsum boldly arched, but bulging somewhat posteriorly. Rostrum minute. Mouth cone unusually long. Mediative process present forming a carina between the 2nd maxillae and maxillipedes, in a more posterior position than in other species.

The males are not in a sufficiently good state of preservation for any details of their reproductive system or internal economy to be made out.

My best thanks are due to Miss E. C. Humphreys for her kind assistance with the figures.